Pedro Mario Pan

AVALIAÇÃO LONGITUDINAL DOS TRANSTORNOS DE HUMOR NA TRANSIÇÃO ENTRE A INFÂNCIA E A ADOLESCÊNCIA: MARCADORES PSICOPATOLÓGICOS, GENÉTICOS E DE NEUROIMAGEM

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina, para obtenção do título de Doutor em Ciências.

São Paulo
2017

Pedro Mario Pan

AVALIAÇÃO LONGITUDINAL DOS TRANSTORNOS DE HUMOR NA TRANSIÇÃO ENTRE A INFÂNCIA E A ADOLESCÊNCIA: MARCADORES PSICOPATOLÓGICOS, GENÉTICOS E DE NEUROIMAGEM

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina, para obtenção do título de Doutor em Ciências.

Orientador:
Prof. Dr. Rodrigo Affonseca Bressan

São Paulo
2017

Pan, Pedro Mario

Avaliação Longitudinal dos Transtornos de Humor na Transição entre a Infância e a Adolescência: Marcadores Psicopatológicos, Genéticos e de Neuroimagem / Pedro Mario Pan. – São Paulo, 2017.
xviii, 152f

Tese (Doutorado) – Universidade Federal de São Paulo. Escola Paulista de Medicina. Programa de Pós-Graduação em Psiquiatria e Psicologia Médica.

Título em inglês: Longitudinal Evaluation of Mood Disorders in the Transition from Childhood to Adolescence: Psychopathological, Genetic and Neuroimaging markers.

1. Crescimento e Desenvolvimento 2.Depressão. 3.Psicopatologia. 4.Coorte. 5. Adolescência. 6.Transtorno Bipolar

UNIVERSIDADE FEDERAL DE SÃO PAULO

ESCOLA PAULISTA DE MEDICINA

DEPARTAMENTO DE PSIQUIATRIA

Chefe do Departamento:

Prof. Dr. Marcelo Feijó de Mello

Coordenadora do Curso de Pós-graduação:

Profa. Dra. Andrea Parolin Jackowski

Pedro Mario Pan

AVALIAÇÃO LONGITUDINAL DOS TRANSTORNOS DE HUMOR NA TRANSIÇÃO ENTRE A INFÂNCIA E A ADOLESCÊNCIA: MARCADORES PSICOPATOLÓGICOS, GENÉTICOS E DE NEUROIMAGEM

Presidente da banca:

Prof. Dr. Rodrigo Affonseca Bressan

Banca Examinadora:

Prof. Dr. Giovanni Salum

Profa. Dra. Sheila Caetano

Profa. Dra. Luccia Valmaggia

Prof. Dr. Nicolas Crossley

Suplentes:

Prof. Dr. Christian Kieling

Prof. Dr. Acioly Lacerda

"Uma teoria é considerada boa se satisfaz dois requisitos: descreve de forma adequada um grande número de observações com base em um modelo que contém apenas poucos elementos arbitrários e faz previsões precisas sobre os resultados de futuras observações"

Stephen Hawking – Uma Breve História do Tempo, 1988.

Dedicatória

À minha esposa Liliana*, por todo amor, apoio e compreensão.*

À minha ***mãe Miriam*** *e ao meu* ***pai Waldir****, pelo amor e pela sabedoria.*

À **Heide** e ao **Dr Chibly** (*in memorian*), *por me receberem como parte da família*

Agradecimentos

Ao professor Dr. Rodrigo Bressan, pelo incentivo e pela inspiração.

Ao professor Dr. Giovanni Salum, pelas palavras de conforto e pelo entusiasmo. Ao professor Argyris Stringaris, por todas as valiosas supervisões.

Às crianças e aos adolescentes avaliados pelo projeto, bem como suas famílias, por toda a paciência e colaboração voluntária.

Aos membros do Departamento de Psiquiatria da Escola Paulista de Medicina – Universidade Federal de São Paulo, pelos 10 anos de acolhimento. Aos membros do INPD, pela oportunidade. À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e ao Conselho Nacional de Pesquisa (CNPq), pelo financiamento deste estudo. Aos membros do *Emotion & Development Branch* do NIH.

Ao meu irmão Lucas, pelo carinho. À toda minha família, amigos e pessoas importantes de Curitiba.

Ao amigo João Daniel, por me acompanhar desde o começo dessa jornada. Aos amigos André e Amanda Gadelha, Ary e Daniela Gadelha, Elson e Gracciele Asevedo, Bruno e Vivian Sini, André e Camila Zugman e Hugo Cogo por me ajudar a lembrar de todas as outras coisas. Aos amigos da resenha esportiva.

Aos amigos do Linc, PROESQ e PRISMA, que colaboraram muito para o meu crescimento.

À Letícia Spíndola, pela caminhada conjunta.

À professora, amiga e sócia Dra. Ana Chaves, pelos conselhos. Ao Ricardo Almeida Prado, pelos *insights.*

Sumário

Lista de Tabelas

Lista de Figuras

Resumo

Objetivo: O maior desafio da psiquiatria é entender a fisiopatologia dos transtornos mentais. O presente trabalho pretende investigar a hipótese pioneira de um padrão alterado de neurodesenvolvimento como um dos componentes causais dos transtornos de humor na transição entre a infância e a adolescência. Os objetivos específicos referem-se aos três estudos realizados nesta tese. Estudo 1: Avaliar se a expressão de genes candidatos é um mediador da relação entre maus tratos na infância e transtorno depressivo. Estudo 2: Avaliar se medidas de conectividade cerebral das regiões que compõem o circuito de recompensa são preditores de depressão na adolescência. Estudo 3: Avaliar a relação entre experiências psicóticas subclínicas e os transtornos mentais, particularmente os transtornos de humor, após 3 anos de seguimento.

Métodos: Os três estudos foram realizados a partir da análise de dados do estudo *High Risk Cohort*. Durante a fase de triagem, avaliaram-se 9937 sujeitos entre 6 e 12 anos provenientes de 57 escolas públicas. Desses, 2511 sujeitos foram submetidos a uma minuciosa avaliação fenotípica cujos instrumentos permitiram o diagnóstico dos principais transtornos mentais. Uma subamostra de 750 sujeitos foi submetida a exame de ressonância magnética do cérebro e a coleta de sangue. Cerca de 80% dos indivíduos inicialmente entrevistados foram reavaliados após 3 anos com os mesmos instrumentos. Estudo 1: A expressão de 12 genes relacionados com o eixo hipotálamo-pituitária-adrenal, com a cascata inflamatória e com o neurodesenvolvimento foi investigada no sangue. A expressão desses genes foi avaliada como um mediador da relação entre maus tratos na infância e depressão. Estudo 2: Foi realizada uma análise por meio de imagens obtidas por ressonância magnética funcional do cérebro no estado de repouso. Modelos de regressão logística avaliaram se a centralidade do estriado ventral, uma medida de intensidade da conectividade com as demais regiões do circuito de recompensa, foi um preditor significante para depressão após 3 anos. Estudo 3: As experiências psicóticas subclínicas foram avaliadas por meio de medidas de autorrelato e de julgamento clínico. Modelos de regressão investigaram se a presença e a intensidade das experiências psicóticas foram relacionadas com os transtornos mentais. Regressão

de *Poisson* testou a relação entre as experiências psicóticas e o número de transtornos mentais comórbidos.

Resultados: Estudo 1: A expressão de genes relacionados com o eixo hipotálamo-pituitária-adrenal e com a cascata inflamatória no sangue de crianças e adolescentes com depressão foi menor quando comparada àquela dos controles saudáveis. A expressão desses genes mediou a relação entre maus tratos na infância e depressão. Estudo 2: A maior centralidade do estriado ventral esquerdo foi um preditor estatisticamente significante para depressão após três anos. Esse efeito não foi encontrado em outros transtornos mentais como ansiedade, uso de substâncias e déficit de atenção e hiperatividade. Estudo 3: As experiências psicóticas subclínicas foram preditores de depressão após 3 anos de seguimento. O número de transtornos mentais comórbidos associou-se às experiências psicóticas em ambas as direções da análise.

Conclusão: Este estudo de coorte permitiu que se investigassem questões fundamentais e altamente inovadoras relacionadas ao entendimento da fisiopatologia dos transtornos mentais. Aspectos psicopatológicos, genéticos e de neuroimagem foram associados ao transtorno depressivo na transição entre a infância e a adolescência. Os resultados encontrados oferecem evidências inéditas sobre o papel da inflamação, do circuito de recompensa e das experiências psicóticas subclínicas na depressão. Os achados podem sugerir alvos específicos para estratégias de identificação precoce e prevenção de transtornos mentais tais como depressão.

Abstract

Objective: To investigate early psychopathological, genetic and neuroimaging aspects of mood disorders in a longitudinal community-based sample of children and adolescents. The specific aims were threefold. Study 1: To investigate candidate gene expression as a mediator for the association between childhood maltreatment and depressive disorder. Study 2: To evaluate the functional connectivity of the ventral striatum within a distributed reward network as a predictor of adolescent depressive disorder after 3 years. Study 3: To explore the bidirectional associations of mood and other common youth mental disorders with psychotic experiences over a 3-year follow-up.

Methods: Study 1: The peripheral blood expression of 12 genes associated with the hypothalamic-pituitary-adrenal axis, inflammation, and neurodevelopment was measured using the real-time polymerase chain reaction. Mediation models were used to test whether differentially expressed genes were significant mediators for the association between childhood maltreatment and depressive disorder. Study 2: Resting-state magnetic resonance imaging assessed the intrinsic functional connectivity within a distributed reward network. Logistic regression models tested whether striatal node strength, a measure of reward-related connectivity, predicted the onset of a depressive disorder over a 3-year follow-up. Study 3: Dimensional and categorical measurements of psychotic experiences were rated by self-report and a trained psychologist. Diagnostic interviews assessed common mental disorders. Assessments were repeated after 3 years. Logistic regression models tested the associations of psychotic experiences and common mental disorders. Poisson regression models investigated whether psychotic experiences predicted the number of comorbid disorders, a proxy for nonspecific “psychiatric load/liability”.

Results: Study 1: Expression levels of genes related to the hypothalamic-pituitary-adrenal axis and inflammation were lower in depressive disorder. Gene expression mediated the effect of childhood maltreatment on the risk of developing depression. Study 2: Increased left ventral striatum node strength predicted increased risk of future depressive disorder (odds ratio 1.54; 95% confidence interval = 1.09-2.18; p = 0.03). Striatal node strength did not predict other common adolescent psychopathological conditions, such as anxiety, attention deficit hyperactivity

disorder, or substance use. Study 3: Bidirectional associations were found between psychotic experiences and youth mental disorders. Baseline psychotic experiences increased the likelihood of any depressive disorder at follow-up. Comorbidity analyses showed significant relationships in both directions, with an increased likelihood of psychotic experiences with greater numbers of comorbid psychiatric disorders at the corresponding time point.

Conclusion: Specific psychopathological, genetic, and neuroimaging characteristics were associated with emergent depressive disorder at the transition from childhood to adolescence. These findings provide novel evidence for involvement of inflammation, the reward network, and psychotic experiences in the pathogenesis of depression, which may lead to specific targets for early identification and preventive strategies.

1 INTRODUÇÃO

1.1 Transtornos Mentais e o Neurodesenvolvimento

Os transtornos mentais lideram a lista de agravos à saúde que causam prejuízo de vida produtiva.[1] A maior parte começa ainda na adolescência e 75% têm seu início antes dos 24 anos.[2] Além disso, o pico do prejuízo funcional ocorre tipicamente nesta mesma faixa etária. Postula-se então que os transtornos mentais, em vez das doenças físicas, é que são as doenças crônicas dos jovens.[3]

Os últimos anos foram marcados por avanços na compreensão dos processos causais das doenças psiquiátricas. Novos métodos de pesquisa proporcionaram o entendimento dos transtornos mentais como doenças do cérebro. A identificação de sinais e de sintomas precoces revelou a importância do período do desenvolvimento cerebral. Diversos estudos convergiram para a formulação de hipóteses que explicam os transtornos mentais como doenças do neurodesenvolvimento.[4, 5] Em um importante editorial publicado no periódico *Nature*, Insel (2010)[6] propõe que a redefinição da esquizofrenia como uma doença do neurodesenvolvimento tem implicações significativas para além da pesquisa científica, pois gera evidências para uma postura mais otimista com os pacientes e renova a esperança para a prevenção da doença nas próximas décadas.

O entendimento atual indica que os transtornos mentais são causados por uma relação complexa entre susceptibilidade genética e fatores ambientais.[7] O impacto da carga genética nas doenças psiquiátricas foi demonstrado inicialmente por meio de estudos em famílias de sujeitos afetados e de estudos de concordância fenotípica entre gêmeos. Determinou-se que a herdabilidade das doenças psiquiátricas varia entre 30% e 90%.[8, 9] Técnicas mais recentes como a varredura completa do genoma (*Genome Wide Association* - GWAS) e a avaliação da expressão gênica proporcionaram a descoberta de determinados mecanismos genéticos que conferem risco de transtornos mentais.[8, 10] Contudo, o risco genético não é específico para um determinado transtorno mental, e ainda não existem marcadores genéticos que tenham algum impacto na prática clínica.[11]

O papel de fatores ambientais nos processos patológicos do comportamento foi identificado desde as primeiras descrições das síndromes clínicas psiquiátricas[12]. O estudo do neurodesenvolvimento apontou para a influência de fatores ambientais

de forma muito precoce, como na gestação e nos primeiros anos de vida.[13] Fatores traumáticos e comportamentos de risco foram associados a uma maior incidência de transtornos mentais.[14] O papel do neurodesenvolvimento foi reforçado a partir das evidências de que determinados fatores ambientais de risco agem de maneira específica durante a infância e a adolescência.[15]

Evidências sugerem que a interação entre o neurodesenvolvimento e os fatores de risco é moderada pela susceptibilidade genética. A investigação desse processo é chamada de estudo de interação gene-ambiente.[15-17] Dessa forma, os transtornos mentais podem ser entendidos como o produto final e um desenvolvimento cerebral atípico resultante de uma cascata complexa de interações entre predisposição genética e fatores ambientais de risco e de proteção. Esta relação é dinâmica e iterativa; apesar de não ser interrompida no início da vida adulta, muito provavelmente ocorre de forma mais acelerada durante o neurodesenvolvimento.[4, 5] A influência dos fatores causais se dá, principalmente, nas fases que antecedem o limiar clínico de identificação dos transtornos mentais, evidenciando-se assim a relevância das fases precoces das doenças psiquiátricas.

1.2 Fases Pré-clínicas dos Transtornos Mentais

O surgimento das hipóteses de padrões alterados de neurodesenvolvimento justificou cientificamente a investigação das fases pré-clínicas dos transtornos mentais. O campo das psicoses foi pioneiro, pois validou prospectivamente o conceito de casos de risco de esquizofrenia a partir de um quadro sintomático atenuado. A contribuição do grupo australiano da *Personal Assessment and Crisis Evaluation Clinic* (PACE)[18] foi essencial para o entendimento das fases precoces da doença. Por meio da investigação retrospectiva de sintomas, Yung et al. (1996)[19] identificaram que pacientes apresentavam sintomas subsindrômicos de psicose antes da eclosão do primeiro episódio psicótico.[18] Foram então propostos critérios clínicos para a definição prospectiva de indivíduos com risco aumentado de psicose, chamados de indivíduos em Estado Mental de Risco (*at-risk mental states*).[20, 21] A abordagem valorizava predominantemente a psicopatologia para determinar os indivíduos em risco. Critérios bem definidos favoreceram a replicação de estudos em diferentes amostras e impulsionaram a avaliação de alterações biológicas anteriores à eclosão do primeiro episódio da doença.

Estudos prospectivos identificaram que indivíduos em Estado Mental de Risco apresentavam taxas de conversão para psicose de 10% a 42% após dois anos de seguimento.[22] O aumento significativo do risco de psicose nesses pacientes promoveu uma série de ensaios clínicos que tinham como desfecho primário evitar a conversão para psicose, ou seja, prevenir a esquizofrenia.[23, 24] Houve diminuição estatisticamente significante nas taxas de transição para psicose nos estudos que avaliaram Terapia Cognitivo-Comportamental (TCC)[25], amissulprida[26], risperidona associada à TCC[27] e Ômega 3.[24] Contudo, a replicação desses achados é necessária, e os primeiros estudos de replicação não encontraram os mesmos resultados anteriores. Um estudo britânico não conseguiu replicar os resultados encontrados com TCC.[28] O ensaio clínico Neurapro não evidenciou superioridade do Ômega 3 em comparação ao placebo nas taxas de conversão para psicose após 6 e 12 meses.[29] Há também um questionamento recente sobre qual deve ser o desfecho primário neste grupo de pacientes, tendo em vista que o prejuízo funcional se estabelece, muitas vezes, por meio de outros sintomas psiquiátricos independentemente dos sintomas psicóticos.[30]

Os estudos que recrutavam pacientes em risco de psicose começaram a registrar altas taxas de comorbidade psiquiátrica e transições para outros transtornos mentais, como depressão e transtorno bipolar, durante o seguimento.[31] A operacionalização dos critérios de risco para psicose impulsionou a elaboração de critérios clínicos de risco específicos para outros transtornos mentais graves, como o transtorno bipolar.[31] Em um estudo retrospectivo, Correll et al. (2007)[32] estudaram as características do período anterior ao Primeiro Episódio de Mania em 51 pacientes jovens (entre 7 e 21 anos). O início dos sintomas prodrômicos foi insidioso, com duração superior a um ano em mais da metade dos casos e duração média de 1,7 ano. Alguns anos depois, Conus et al. (2010)[33] identificaram três grupos principais de sintomas: alterações no ciclo sono-vigília, sintomas subsindrômicos de humor e outras alterações inespecíficas do comportamento.

1.3 A Reaproximação com o Paradigma Médico

Os achados de alterações biológicas precedentes ao início dos quadros psicóticos permitiram a reaproximação do campo da psiquiatria com o paradigma médico de prevenção. O desenvolvimento da epidemiologia e de métodos estatísticos

para o controle de “confundidores” permitiu a associação de fatores de risco com doenças mesmo após longos períodos de latência, como ocorreu com tabagismo e câncer de pulmão.[34] Na cardiologia, por exemplo, fatores comportamentais (p. ex. sedentarismo) e biológicos (p. ex. dislipidemia) foram incorporados nas avaliações clínicas de rotina por seu efeito preditivo para desfechos graves, como a morte por infarto do miocárdio. Assim, o foco do tratamento tornou-se o fator de risco e não mais a doença, permitindo a prevenção do desfecho negativo.[7]

A psiquiatria também se aproximou do paradigma médico por meio das propostas de estadiamento dos quadros psiquiátricos.[35, 36] Foram propostos estadiamentos clínicos embasados em sintomas e em evidências preliminares de progressão neurobiológica, da mesma forma que ocorre em áreas como a oncologia. Nessa especialidade médica, o estadiamento proporcionou uma mudança radical na prática clínica. Estabeleceu-se rastreio e identificação precoce em escalas populacionais, e os tratamentos foram ajustados de acordo com o acometimento fisiopatológico.[37] Uma percepção mais esperançosa proporcionou a diminuição do estigma. A determinação dos fatores de risco permitiu a prevenção.[38] Os avanços descritos na oncologia são o resultado de investigações científicas em estudos longitudinais com grande tamanho amostral.

1.4 Neurociência Populacional

A pesquisa em neurociência incorporou alguns dos conceitos do paradigma médico em uma abordagem metodológica chamada de neurociência populacional (*populational neuroscience*).[7, 39] Como sugere Paus (2010)[39], entender os processos cerebrais subjacentes às doenças do cérebro, assim como suas causas, é fundamental para alcançar o principal objetivo de longo prazo da neurociência populacional: intervenções preventivas individualizadas (*personalized preventive medicine*).

O objeto de estudo da neurociência populacional é o conjunto de variáveis coletadas em estudos longitudinais que avaliaram grandes amostras comunitárias. A integração das principais fontes de dados – *genoma* e *epigenoma*, *ambientoma (*fatores de risco ambientais) e *fenoma* (desfechos fenotípicos) – proporciona ao pesquisador a possibilidade de testar suas hipóteses. O estado da arte de cada disciplina é utilizado para tratar e analisar as variáveis coletadas. Os dados já

processados pelos especialistas de cada área (genética, epidemiologia, neurociência cognitiva, neuroimagem, psicometria, psiquiatria) são integrados a grandes repositórios de dados elaborados e mantidos por equipes multidisciplinares. Assim, as perguntas de pesquisa são avaliadas em sua pertinência perante à literatura e testadas no "laboratório virtual" da neurociência populacional: o banco de dados.

As amostras dos estudos de neurociência populacional precisam satisfazer três critérios principais: tamanho amostral suficiente, representatividade da população estudada e desenho longitudinal. A complexidade do cérebro e dos diversos fatores de risco aponta para a ausência de uma via causal única dos transtornos psiquiátricos, seja genética ou ambiental.[11, 12, 16] Em vez disso, inúmeros estudos demonstraram que os fatores de risco ambientais e os marcadores genéticos explicam somente uma parte dos desfechos comportamentais. Assume-se, assim, que o efeito de cada variável preditora é pequeno e, portanto, são necessárias grandes amostras para encontrar tais efeitos.

Ainda, as amostras devem representar, em algum grau, a população em que estão inseridas. Não se pode, por exemplo, recrutar sujeitos exclusivamente com base em diagnósticos já estabelecidos. Esse critério é importante para identificar as trajetórias normais, um pressuposto para entender os casos com trajetórias diferentes. Dessa forma, tanto fatores de risco para as trajetórias atípicas como fatores de proteção para as trajetórias normais podem ser investigados. Modelos estatísticos que controlam ou exploram a interação de características individuais, como marcadores genéticos, são utilizados para investigar os fatores de risco e de proteção nos desfechos de interesse do pesquisador.

Por fim, um dos critérios fundamentais para se estabelecer causalidade é a avaliação longitudinal, preferencialmente prospectiva, das associações entre os fatores de risco investigados e os desfechos de interesse.[40] O desenho longitudinal prospectivo diminui vieses nas associações encontradas em estudos transversais ou retrospectivos; pode, também, determinar a direção das associações encontradas, um aspecto fundamental para se determinar causalidade.

1.5 As Trajetórias dos Transtornos Mentais

Os estudos com desenho longitudinal também permitem avaliar a trajetória dos sintomas psicopatológicos e dos transtornos mentais.[41] Nesse tipo de estudo, as

manifestações e os diagnósticos realizados nas primeiras avaliações podem evoluir de forma homotípica ou heterotípica. A manutenção do mesmo diagnóstico realizado nas primeiras avaliações é chamada de evolução homotípica. A evolução para transtornos diferentes daqueles apresentados na avaliação inicial é chamada de evolução heterotípica.[42]

Contudo, a definição mais precisa dos desfechos fenotípicos ainda é um dos grandes desafios no estudo da evolução longitudinal dos transtornos psiquiátricos. Ainda não há definições com base em substratos biológicos. As classificações diagnósticas continuam determinando os transtornos mentais por intermédio de agrupamentos de sintomas. O resultado é a manutenção de uma hierarquia historicamente estabelecida; por definição, um transtorno psicótico é mais grave que – ou seu diagnóstico deve ser priorizado frente a – um transtorno de humor ou de ansiedade, por exemplo.[43-45] Outra consequência é o excesso de transtornos comórbidos, que significa o enquadramento dos sintomas em diversas classes diagnósticas simultaneamente.[46] Isso é particularmente relevante na psiquiatria do desenvolvimento.[47, 48]

1.6 Abordagens Dimensionais para a Psicopatologia

Os sintomas comportamentais que antecedem os transtornos mentais muitas vezes não preenchem critérios de uma categoria diagnóstica específica. Outras vezes falham em atingir o limiar diagnóstico por não alcançar os critérios de duração ou de prejuízo funcional. Além disso, na outra ponta do problema, os sintomas psiquiátricos podem gerar o preenchimento do critério de diversos transtornos comórbidos. A evolução heterotípica ou homotípica, consequentemente, não abrange completamente a diversidade de apresentações de sintomas. Assim como o risco genético e os fatores de risco ambientais, as alterações comportamentais que precedem os quadros podem ser polimórficas e inespecíficas. Evidenciou-se assim a necessidade de uma abordagem para a avaliação psicopatológica do neurodesenvolvimento que fosse independente da estrutura das categorias propostas pelas classificações diagnósticas; deveria, ainda, englobar todo o espectro de apresentação de uma determinada emoção ou comportamento, das manifestações leves até as mais graves. Essa abordagem é chamada de psicopatologia dimensional e foi amplamente utilizada na psiquiatria do desenvolvimento.[48-51] A edição mais

recente do manual americano de classificação diagnóstica já propõe a avaliação dimensional de alguns aspectos de comportamento.[45]

A avaliação dimensional dos sintomas psiquiátricos proporcionou a investigação de grupos de comportamentos e emoções independente de um diagnóstico categorial. Os mecanismos cerebrais subjacentes a determinadas dimensões de sintomas foram investigados em estudos de neuroimagem. Em concomitância, os mesmos métodos foram utilizados para a avaliação de comportamentos em indivíduos saudáveis, estabelecendo o que seria o funcionamento cerebral típico. Estruturas cerebrais e padrões de ativação cerebral foram associados a sintomas e comportamentos específicos.[5, 52, 53] Iniciativas recentes foram desenvolvidas a partir desse conceito, como o *Research Domain Criteria* (RDoC).[47, 54, 55]

Na direção proposta pelo RDoC, Pan et al. (2013)[56] avaliaram uma abordagem dimensional para os sintomas de mania na infância e no início da adolescência. Dentre as principais conclusões, destaca-se a confirmação da estrutura latente dimensional de sintomas de mania, com as dimensões previamente encontradas "*Under-control*" e "*Exuberant*". A técnica de Análise de Classes Latentes permitiu a identificação de um grupo de pacientes com alta carga de sintomas de mania. O grupo era composto de pacientes gravemente comprometidos, com níveis significativos de comorbidades psiquiátricas e prejuízo funcional. A dimensão "*Under-control*" foi associada a impacto no funcionamento mesmo após o controle de potenciais confundidores, como a ocorrência de transtornos psiquiátricos comórbidos. Ainda, indivíduos da classe latente mais gravemente comprometida apresentaram uma prevalência elevada de história familiar de transtornos de humor, comparável a amostras clínicas de transtorno bipolar.

As situações clínicas em que categorias diagnósticas não conseguem explicar a relação entre sintomas, impacto funcional e sofrimento são muito frequentes e ultrapassam os transtornos de humor. O apêndice da tese traz um artigo que propõe a validação de um fator latente de psicopatologia geral, englobando diversos agrupamentos de sintomas psiquiátricos. Esse fator, denominado Fator P, seria responsável pelo componente do impacto e do sofrimento gerado pelos sintomas que não é explicado por diagnósticos específicos. O Fator P é estimado por meio de métodos matemáticos chamados de Modelagem de Equação Estrutural[57] e representa o componente que é ignorado pelas classificações categoriais, mas que é percebido pelo clínico em sua prática diária. O artigo investiga as relações do Fator P

com aspectos neuropsicológicos, como a função executiva, e fatores familiares, utilizando a psicopatologia parental.

1.7 Lacunas do Conhecimento em Neurodesenvolvimento dos Transtornos Mentais

As últimas décadas foram marcadas por importantes descobertas científicas sobre os fatores biológicos e ambientais associados aos transtornos mentais. Houve, contudo, pouco avanço na tradução desse conhecimento em benefícios reais para a vida dos pacientes. O diagnóstico em psiquiatria ainda é eminentemente clínico, pois faltam ferramentas diagnósticas mais precisas como marcadores de progressão e de risco. As classificações diagnósticas reportam-se a constructos criados há mais de cem anos.[54, 58] Há uma escassez de novos tratamentos, e as intervenções disponíveis para o clínico de saúde mental são limitadas. O estudo das trajetórias típicas e atípicas de desenvolvimento proporcionou a identificação das fases pré-clínicas dos principais transtornos psiquiátricos. Todavia, ainda não foi suficiente para a identificação precisa em nível populacional de jovens em risco de manifestar os transtornos mentais mais comuns, como os transtornos de humor.[47, 55] Assim, determinar os aspectos do neurodesenvolvimento implicados na etiologia dos transtornos mentais poderá sugerir novos caminhos para superar a estagnação do campo.[4, 5, 7, 39, 52, 54, 58-60] O presente trabalho pretende investigar marcadores precoces dos transtornos mentais durante a fase de transição da infância para a adolescência, particularmente aqueles relacionados com potenciais vias etiológicas dos transtornos de humor.

1.8 Apresentação da Tese

A partir da introdução, a tese será apresentada em inglês e na forma de artigos científicos. Serão apresentados os objetivos da tese e uma descrição geral da metodologia empregada. A conclusão trará uma síntese dos achados dos estudos desenvolvidos. Cada artigo explorará um aspecto específico da interface entre os transtornos de humor e marcadores psicopatológicos (sintomas psicóticos), ambientais (trauma precoce) e de neuroimagem (circuito de recompensa). Os artigos incluem introdução e revisão de literatura específica para a área de conhecimento investigada, seguidas de objetivos, metodologia, resultados e discussão dos achados.

1.8.1 Apresentação da Metodologia

A presente tese apresentará resultados encontrados a partir do estudo de coorte chamado Coorte de Alto Risco para o Desenvolvimento de Transtornos Mentais e Resiliência (*High Risk Cohort* - HRC*).* O estudo HRC faz parte do edital Institutos Nacionais de Ciência e Tecnologia (INCTs), por meio do projeto Instituto Nacional da Psiquiatria do Desenvolvimento (INPD).

O estudo HRC investiga os aspectos do neurodesenvolvimento envolvidos nos transtornos de saúde mental na infância e na adolescência. A metodologia empregada será minuciosamente descrita na seção 4 – *Methods*. Sua amostra insere-se no paradigma da neurociência populacional, visto que satisfaz os critérios previamente elencados: tamanho amostral suficiente, representatividade da população estudada e desenho longitudinal.

1.8.2 Estudos Realizados

O artigo 1 investigou a associação entre trauma precoce e a expressão sanguínea de genes candidatos na depressão. Foram usados dados coletados sobre ocorrência de abuso físico e emocional, de maus-tratos e de negligência como preditores de depressão na infância e no início da adolescência. O papel da expressão de genes previamente associados ao transtorno depressivo foi investigado por meio de modelos de mediação. Os genes candidatos estudados foram escolhidos a partir de uma revisão da literatura e da confirmação de sua expressão no tecido disponível (sangue). A mediação é determinada quando a associação entre duas variáveis é parcialmente explicada por uma terceira variável ou por um grupo de variáveis chamadas mediadoras. Assim, o artigo 1 investiga se a associação entre trauma precoce e depressão é mediada pela expressão sanguínea de genes previamente associados ao transtorno depressivo. O artigo 1 foi publicado no periódico científico *Journal of Psychiatric Research* em 2017.

O artigo 2 avaliou aspectos da conectividade cerebral de uma rede específica previamente associada aos transtornos de humor: a rede de recompensa. Utilizando-se de imagens obtidas por ressonância magnética funcional em repouso (*resting-state Magnetic Resonance Imaging - rs-MRI*), avaliou-se a correlação entre medidas indiretas de fluxo sanguíneo cerebral (*Blood-oxygen-level Dependent contrast imaging* - BOLD) em áreas implicadas no processamento de recompensas comportamentais. Estabeleceu-se assim medidas da conectividade funcional de cada região, chamadas

de nós (*nodes*), da rede de recompensa. O foco principal da avaliação foi a região do estriado ventral, que tem papel fundamental nesta rede. As medidas de conectividade funcional do estriado ventral foram então utilizadas como preditores de depressão após 3 anos de seguimento. O artigo 2 foi aceito para publicação no periódico *American Journal of Psychiatry.*

O artigo 3 explorou a associação entre experiências psicóticas atenuadas e a ocorrência de transtornos mentais na transição entre a infância e a adolescência. O desenho longitudinal permitiu a investigação da direção dessa associação, ou seja, quais transtornos mentais precedem e quais são precedidos por experiências psicóticas no decorrer de um período de 3 anos. O interesse inicial residia particularmente na associação com os transtornos de humor e os transtornos psicóticos. Contudo, o artigo traz também uma exploração mais ampla da associação entre as experiências psicóticas e os transtornos mentais mais comuns nessa faixa etária. O artigo 3 será submetido para o periódico *Schizophrenia Bulletin*.

2. OBJECTIVES

2.1 Main

The main objective of this thesis was to investigate early psychopathological, genetic, and neuroimaging aspects of mood disorders in a longitudinal community-based sample of children and adolescents.

2.2 Specifics

2.2.1 Study 1

To investigate the gene expression of 12 genes related to the hypothalamic-pituitary-adrenal axis, inflammation, neurodevelopment, and neurotransmission in the blood of children and adolescents as a mediator for the association between childhood maltreatment and depressive disorder.

2.2.2 Study 2

To evaluate the functional connectivity within a distributed reward network, assessed using resting-state functional magnetic resonance imaging (r-sfMRI), as a predictor of adolescent depressive disorder after 3 years.

2.2.3 Study 3

To explore the bidirectional associations of mood and other common youth mental disorders with psychotic experiences in a community-based longitudinal sample of children and adolescents.

3. METHODS

3.1 Study Design

Subjects enrolled in this study participate in large community school-based survey, the High-Risk Cohort (HRC), which has the objective to map neurodevelopmental trajectories in typical development and in common mental illnesses.[61, 62] We selected two subgroups from an initial pool of almost 10,000 subjects: a random group and a high-risk group. The high-risk group was based on the presence of psychiatric symptoms in the index child and high family loading of psychopathology assessed by the screening interview. We used this strategy to increase the incidence of mental disorders in this group, in order to allow suitable power to study trajectories of atypical neurodevelopment.

3.1.1 Screening Phase

In the screening phase, we assessed child and family member symptoms of psychiatric disorders in a screening interview. Our study population was composed by 6-12 years-old students from 57 public schools in two large Brazilian metropolitan areas (Porto Alegre city and São Paulo city). We invited biological parents to participate during the school registry day. Parents provided consent and information about the child. Lay interviewers conducted the Family History Survey (FHS).[63] We evaluated 9,937 children. The biological mother was the main informant in 88% of the cases. From this pool, we used a simple randomization procedure in order to select subjects for the random-selection group (n=958). Selection for the high-risk stratum involved a risk-prioritization procedure, focused on individuals with high individual and familial load of psychiatric symptoms (n=1,553).

3.1.2 Baseline Assessment

The baseline assessment comprised household and school visits. Participants reported on an extensive research protocol. We will now describe the procedures of each study visit.

3.1.3 Household Interview

The Development and Well-Being Assessment (DAWBA) was used to assess diagnosis of DSM-IV psychiatric disorders.[64] DAWBA is a validated structured

interview administered by trained lay interviewers. The Brazilian Portuguese version shows appropriate psychometric proprieties and high inter-rater reliability.[65] Diagnostic reliability for the rating procedure retrieved acceptable indexes of inter-rater agreement. Kappa values ranged from 0.72 for hyperkinetic disorders to 0.84 for anxiety and mood disorders. DAWBA integrates verbatim responses to open-ended questions in order to supplementing information from structured questions.

We obtained data on psychopathology from biological parents of 2,511 subjects in a household interview. The main caregiver of the child was the informant (biological mother in 91.5% of the cases). The interviewers attended to extensive training and supervision using real patients' video reports. During the field assessment period, the research group provided constant supervision and discussion. Based on the information obtained by the interviews, diagnoses were assigned by trained psychiatrists. Rater's training consisted in several meetings held by an experienced child psychiatrist, who was also a well-experienced rater of DAWBA.

As part of the household interview, the respondent parent answered the Mini International Psychiatric Interview (MINI).[66, 67] The Brazilian version of MINI has shown adequate inter-rater reliability and satisfactory psychometric characteristics when compared to standard structured diagnostic interviews, as CIDI and SCID.[67] Parents also reported on an extensive protocol encompassing demographics, child prenatal and perinatal information, and environmental risk factors, such as early life stressors and trauma. Finally, parents reported on two dimensional measures of psychopathology: the Strength and Difficulties Questionnaire (SDQ)[68] and the Child Behavior Checklist (CBCL).[50]

3.1.4 School Child Interview

Children were evaluated in school by trained psychologists and speech therapists. The psychologist interview and testing protocol included specific neurocognitive tests, child evaluation of psychotic experiences, anxiety, temperament, and risk factors. The speech therapists examination comprised an evaluation of literacy and phonological awareness.

3.1.5 Neuroimaging and Blood Biomarkers

Subjects who completed household and school evaluation were eligible to participate in the Neuroimaging and Blood Biomarkers phase. We aimed to invite 750

subjects to participate from the pool of 2511 children. We acquired brain magnetic resonance imaging (MRI) in each study city using 1.5 Tesla General Electric Scanner (GE Signa HDX and GE Signa HD—G.E., USA). The following sequences were acquired: (a) high-resolution tridimensional T1-weighted; (b) diffusion tensor imaging (DTI); (c) intrinsic connectivity fMRI; (d) MT ON/OFF. We asked subjects to fixate on a target during resting-state intrinsic connectivity acquisition. We successfully acquired at least one MRI sequence from 741 subjects.

Blood samples were available from 625 subjects. Blood draw times ranged between 10 a.m. and 4 p.m. Blood samples were processed in few hours and serum was stored at −80 °C.

3.2 Follow-up Assessment

We re-contacted parents 3 years after the first evaluation. The first follow-up evaluation included two household visits. First, a lay interviewer assessed parents or main caregivers of study subjects. Second, psychologists interviewed the adolescent with the self-reported version of the protocol.

Attrition rates were in the acceptable range of approximately 20%. We were unable to contact any family member in 10.2% (n=255) of cases. In 9.8% (n=246) of the cases the main caregiver refused to participate. Therefore, the follow-up sample consisted of 2010 subjects (80.05% from baseline household sample). Factors associated to attrition by loss of contact or refusal were: (a) lower maternal education (chi-square 14.07, p<.001) and lower socioeconomic status (chi-square 6.24, p<.05), and being from Porto Alegre City site (chi-square 4.57, p<.05). Any anxiety disorder at baseline (chi-square 9.754 p<.01) increased the chance of retention.

3.2.1 Follow-up Household Parental Interview

Parents reported on a household interview conducted by trained lay research assistants. We kept the main structure of the baseline parental protocol, adding questions related to adolescent specific behaviors, such as alcohol and drug use. We avoided to recollect time invariant measures. We used a life-chart to only ascertain the period between the two interviews. We adopted this approach to diminish burden for the interviewed parent.

3.2.2 Follow-up Household Adolescent Interview

Trained certified psychologists interviewed the subjects in their houses in one session. Interview protocol included neurocognitive tests and the same child evaluation of psychotic experiences. We have also collected information of risk factors, violence, alcohol, and drug use. In the 3-year follow-up evaluation, we introduced the DAWBA self-report version. Trained psychiatrist raters evaluated parent- and self-reported DAWBA information to ascertain DSM-IV psychiatric diagnoses. They used a digital platform to integrate both sources of information. Research team met frequently to discuss inconclusive cases. At follow-up, raters were blinded for baseline psychiatric disorders.

3.3 Ethics

The study was approved by the Ethics Committee of the Universidade de São Paulo (USP)(CAAE 0009.1.015.015-09 "*Parecer Consubstanciado*" 1138/08) and Universidade Federal de São Paulo (UNIFESP)(CAAE: 06172912.6.1001.5505 "*Parecer Consubstanciado*" 87075/12 and CAAE 65824817.3.0000.5505 "*Parecer Consubstanciado*" 0268/2017). It was also submitted and approved by the National Council of Health Registry (CONEP)(Registry number 15.457). Parents provided written informed consent for all participants. Children provided verbal informed assent; whenever possible, children also provided written consent. We follow the ethical instructions and considerations from Brazilian governmental agency *Conselho Nacional de Saúde* (CNS) from the Ministry of Health, represented by the document "*Resolução Normativa 466/12*". It encompasses the main ethical principles that guide best practices in medical research in humans. We attached a copy of the written consent and a copy of the latest Institutional Review Board approval in the end of the thesis.

We invited all families for an appointment with a trained psychologist and social worker in case they were interested in receiving the results of the evaluations from the research protocol. We children referred for clinical evaluation any participant identified as being under the need of care. We also referred specific situations involving serious risk of physical or psychological harm to competent governmental authorities according to Brazilian Ministry of Health guidelines.

4. RESULTS AND DISCUSSION

We will present the results and discussion of the thesis in three scientific papers. The HRC study provided data to investigate the specific objectives of the thesis. Each one will be addressed by a scientific paper. The structure of the papers are the following: abstract, introduction and review of the literature, methodology, results and discussion.

4.1 Study 1: Gene expression in blood of children and adolescents: mediation between childhood maltreatment and major depressive disorder-

This paper was published in the Journal of Psychiatric Research.
Impact Factor - 4.18 – 2016 Journal Citation Reports.(69)
ISSN - 0022-3956 - Qualis A1, MEDICINA II (2013-2016)

4.1.1 Authors and Abstract

Leticia Maria Spindola, MSc[1,2,3,4,a]; Pedro Mario Pan, MD MSc[2,3,4,a]; Patricia Natalia Moretti, PhD[1,2,3,4]; Vanessa Kiyomi Ota, PhD[1,2,3,4]; Marcos Leite Santoro, PhD[1,2,3]; Hugo Cogo-Moreira, PhD[2,3,4]; Ary Gadelha, MD PhD[2,3,4], Giovanni Salum, MD PhD[3,8]; Gisele Gus Manfro, MD PhD[3,8]; Jair Jesus Mari, MD PhD[2,3,4]; Helena Brentani, MD PhD[3,5]; Rodrigo Grassi-Oliveira, MD PhD[6,7]; Elisa Brietzke, MD PhD[4]; Euripedes Constantino Miguel, MD PhD[3,5]; Luis Augusto Rohde, MD PhD[3,8]; João Ricardo Sato, PhD[3,9]; Rodrigo Affonseca Bressan, MD PhD[2,3,4]; Sintia Iole Belangero, PhD[1,2,3,4*]

[a] These authors contributed equally to this work.

1 Genetics Division, Department of Morphology and Genetics, Universidade Federal de Sao Paulo (UNIFESP), Brazil;

2 LiNC - Interdisciplinary Laboratory of Clinical Neurosciences, UNIFESP, Brazil;

3 National Institute of Developmental Psychiatry for Children and Adolescents (INPD), Brazil;

4 Department of Psychiatry, UNIFESP, Brazil;

5 Department & Institute of Psychiatry, Faculdade de Medicina da Universidade de São Paulo, Brazil;

6 Post-Graduation Program in Psychology, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil;

7 Developmental Cognitive Neuroscience Lab, PUCRS, Brazil;

8 Department of Psychiatry, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Brazil;

9 Center of Mathematics, Computation and Cognition, Universidade Federal do ABC, Brazil.

Investigating major depressive disorder (MDD) in childhood and adolescence can help reveal the relative contributions of genetic and environmental factors to MDD, since early stages of disease have less influence of illness exposure. The differentially expressed genes were inserted in a mediation model in which CM, MDD, and gene

expression were, respectively, the independent variable, outcome, and intermediary variable. *NR3C1*, *TNF*, *TNFR1* and *IL1B* were expressed at significantly lower levels in the MDD group than in the other groups. CM history did not exert a significant direct effect on MDD. However, an indirect effect of the aggregate expression of the 4 genes mediated the relationship between CM and MDD. In the largest study investigating gene expression in children with MDD, we demonstrated that *NR3C1*, *TNF*, *TNFR1* and *IL1B* expression levels are related to MDD and conjunctly mediate the effect of CM history on the risk of developing MDD. This supports a role of glucocorticoids and inflammation as potential effectors of environmental stress in MDD.

Keywords: Major depressive disorder, gene expression, Inflammation, glucocorticoids, childhood maltreatment, child

4.2.2 Introduction

Major Depressive Disorder (MDD) is a complex psychiatric disorder. Genetic factors contribute up to 40% of MDD risk and are complemented largely by individual-specific environmental exposure to adverse life events.[1] Exposure to early adverse life events, including childhood maltreatment (CM), substantially increases risk for several psychiatric disorders in both children and adults.[2-4] Among mood disorders, CM has been also associated to MDD worse clinical and treatment outcomes.[5] Thus, a better understanding of the biological mechanisms by which CM confers MDD risk might shed light on novel ways to prevent and treat MDD.

Hyperactivity of the HPA axis is a consistent finding in MDD.[6] Several studies have described a reduced function, or resistance, of glucocorticoid receptor (GR), encoded by NR3C1 gene, in patients with MDD.[7, 8] A second biological system involved in MDD etiology is inflammation.[9] mRNA levels of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, are higher in MDD patients than in controls.[10, 11] Moreover, proinflammatory cytokine system activation might function in the aforementioned HPA-axis hyperactivity, since considerable data show that TNF-α induces GR resistance.[12, 13] Other potential mechanisms that might contribute to MDD pathogenesis are disturbances of neurodevelopment; this hypothesis is based on clinical evidence that MDD might be related to structural abnormalities in various brain regions.[14, 15] Moreover, studies suggest that other neurotransmitter systems, in addition to the monoamine neurotransmission, contribute to the pathophysiological mechanisms of mood disorders.[16] Glutamatergic abnormalities have been implicated in the pathophysiology of MDD and it has been reported an increase of glutamate levels in serum/plasma of MDD patients compared to controls.[17, 18]

A useful method to investigate the pathogenesis of this disorder is the use of peripheral blood to measure the expression levels of genes. As there is a clear association between the immune system and MDD, the development of the disorder might be associated with some systemic alterations[9], which can be captured by gene expression in blood. Moreover, it has observed that the correlation between transcripts present in whole blood and brain tissues was around 0.5, showing that transcriptome in blood is neither perfectly correlated nor uncorrelated with that in many brain regions.[19] Furthermore, peripheral blood is an accessible tissue that, via low-invasive

procedures, can be used to evaluate several biomarkers, for example mRNA levels or proteins, using quantitative techniques.

The mRNA levels in blood of MDD-associated genes have been widely reported to differ between MDD patients and healthy controls (see [20] for review). A recent study in a larger cohort of depressed patients has found 129 genes associated with current MDD, that were enriched for IL-6-signaling and natural killer cell pathways.[21] However, some findings have not been replicated. Several factors could underlie the inconsistency, such as gender and age, as well as the clinical heterogeneity of MDD itself.[22, 23] These findings might be related to the effects of illness duration, number of recurrent episodes, ongoing or previous medication and lifestyle factors. In this context, studies focused on investigating gene expression in children and adolescents with MDD, a group with potentially shorter illness exposure[24], might overcome the aforementioned limitations and help further elucidate the mechanisms underlying early MDD.

In this study, we had two main goals: 1) to compare mRNA levels of 12 genes related to HPA axis (NR3C1 and FKBP5), inflammation (TNF, TNFR1, TNFR2 and IL1B), neurodevelopment (DISC1, PDE4B and QKI) and neurotransmission (SLC1A4, GLUL and COMT) in blood of children and adolescents with: a) MDD (MDD group); b) high levels of depressive symptoms but without MDD (DS group); and c) healthy controls (HC group); 2) to employ a mediation model to verify if childhood maltreatment history, an environmental stressor trait that influences MDD risk, affects MDD through the expression of genes, specifically the differentially expressed genes (DEGs) from Aim 1. To our knowledge, this is the first study to test a mediation model of CM history, gene expression and MDD in children and adolescents from a non-medicated community sample. Furthermore, this is the largest study to examine gene expression in blood of children with MDD.

4.2.3 Methods and Materials

Study procedures

We selected a subsample from a large prospective community school-based study in Brazil, the High Risk Cohort Study for Psychiatric Disorders. The cohort characteristics and study design are detailed elsewhere[25] and Supplementary Materials and Methods contains additional information. Briefly, baseline assessment

was performed in multiple visits including a household parent interview (n=2,512) and, on a separate visit, collection of blood samples to assess peripheral biomarkers (n=625). In the household parent interview, participants were assessed using the structured diagnostic interview Development and Well-Being Assessment (DAWBA)[26] to evaluate psychiatric diagnosis according to the DSM-IV. Given that there was a gap between DAWBA evaluation and blood collection, in the same day that blood was collected, psychopathology measures were assessed using Child Behavior Checklist (CBCL).[27] The Research Ethics Committee approved the research protocol. Parents provided written informed consent. We obtained verbal assent from children and adolescents. Youngsters who were able to read, write and understand provided written consent.

Participant selection for RNA expression study

For this study, from the subsample of 625 subjects, we selected all children with MDD according to DSM-IV criteria (using DAWBA) to comprise the MDD group. We further selected all children who did not fulfill MDD criteria according to DAWBA DSM-based rating. This group presented high levels of depressive symptoms according to the CBCL DSM-Oriented affective problem scale (scores ≥10), a dimensional measure of affective psychopathology. Therefore, DS comprised children with depression symptoms but without MDD diagnosis. This threshold was chosen based on a study that investigated the correspondence between CBCL DSM-Oriented scales with clinical diagnoses in a clinic sample of children and adolescents.[28] We created DS group with the intention of having a group with depression symptoms but without MDD diagnosis. Lastly, a comparison group of typically developing children or “healthy controls” was selected from the 625 children. We analyzed the blood expression of the selected genes in all individuals from our sample who met the following criteria: i) no DSM-IV disorder in DAWBA assessment; ii) affective-problem score of 0 in CBCL DSM-Oriented scale; and iii) no depression, mania or suicide attempt in biological parent (according to Mini International Psychiatric Interview in household interview[29]). Finally, we also excluded children from all groups who had used any psychiatric medication in the month before blood collection.

Therefore, we evaluated 20 children and adolescents with MDD, 49 children and adolescents without MDD but with high levels of depressive symptoms and 61 controls.

History of CM assessment

Parents and children answered four questions about history of adverse environment and trauma, which represent the following CM categories: (a) physical abuse (infliction of bodily injury by non-accidental means); (b) neglect (failure to provide minimum care and/or the lack of supervision); (c) emotional maltreatment (persistent and extreme thwarting of a child's basic emotional needs) and (d) sexual abuse (sexual contact or attempted contact for purposes of sexual gratification or financial gain).[30] We used Confirmatory Factor Analysis to evaluate a latent model (an environmental trait) encompassing all questions from both, self and parental report. Data showed excellent fit for a second-order model (for more information, see[3]). For the purposes of this study we used saved factor scores as observed continuous variable in the mediation analysis (table S1).

Genetic analysis

For information about blood sample preparation, see Supplementary Materials and Methods. We measured the whole blood mRNA levels of the candidate genes playing a role in four biological systems associated with MDD pathogenesis, as mentioned above. We selected the genes based on a review of the literature considering the following aspects: (i) at least one report on association with MDD; (ii) plausibility of their involvement in MDD pathophysiology; and (iii) expression in blood according to information in the Anatomy tool of GENEVESTIGATOR.[31] Genes that presented medium to high mRNA levels in blood in this tool were defined as expressed in whole blood. For more information about the target gene selection are given in Table S2. Gene-expression analysis is described in Supplementary Materials and Methods and PCR assays and targeted exons and transcripts are detailed in Table S3.

Gene expression was quantified using the relative threshold (Crt) method with the geometric mean (GM) between GAPDH and ACTB gene expression as endogenous control. ΔCrt values (ΔCrt = Crttarget gene - CrtGM GAPDH/ACTB) were calculated for each sample. We have tested ACTB and GAPDH genes in blood samples and found a strong correlation between them and no association with MDD diagnosis and psychopathology symptoms. Moreover, we used the NormFinder algorithm for identifying the optimal normalization gene among our set of candidate genes and, among all genes, ACTB and GAPDH presented the smallest stability values.[32]

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0. We performed one-way ANOVA to verify if there were differences among the three groups regarding age, CBCL total, CBCL DSM-oriented and CM history scores. Gender and site (Porto Alegre or Sao Paulo) effects were tested using the chi-squared test. We also used chi-squared test to verify if there were differences between MDD and DS groups regarding the presence of any psychiatric comorbidities.

Gene expression differences among MDD, DS and HC groups

The general linear model (GLM) was used to compare the ΔCrt values among the groups (MDD, DS and HC). We used gender and site as fixed factors in the analyses. Furthermore, we used the Bonferroni correction for multiple comparisons, considering significant p-values < 0.0042 (α / m, where α = 0.05 and m = 12 genes tested). The fold was calculated by -1 / f, where f = 2-ΔCrt mean of MDD group / 2-ΔCrt mean of reference group (DS or HC). We also assessed the specificity of our analysis changed the outcome to the co-morbid disorders (deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD) and anxiety disorder). Moreover, the differentially expressed genes (DEGs) from the comparison among the three groups were compared between children of MDD group with CBCL DSM-oriented affective score ≥ 10 (subset of MDD group, n = 10) and healthy controls using the GLM.

Mediation model with the DEGs

We applied a parallel multiple mediation analysis that links a putative cause (CM history) to a presumed effect (have MDD) at least in part via intermediary variables (gene expression) (figure 1). We tested this model using the PROCESS macro for SPSS [33]. We used as mediator variables only the ΔCrt values of DEGs from our first aim. Although differential expression among groups is not essential for using a gene as a mediator variable, we used only the DEGs in order to reduce the number of variables to be included in the model. Indirect effects were estimated using the bootstrap bias corrected method that generates 95% confidence intervals (CIs). CIs that do not include zero indicate statistically significant direct or indirect effects. We used 10,000 bootstrap samples for the analyses, which were performed only with MDD

and HC groups because the PROCESS outcome variable must be dichotomous or quantitative, and our main goal was to compare participants with the disorder (MDD) against those without (HC). Site (Porto Alegre or Sao Paulo) was used as covariate in the analysis. We did not use gender as a covariate for the mediation model because, considering our sample size, the number of variables in the model could lead to an overfitting problem. We also assessed the specificity of our mediation model changed the outcome (have MDD) to CD or ODD, that are externalizing psychiatry disorders associated with CM.[34]

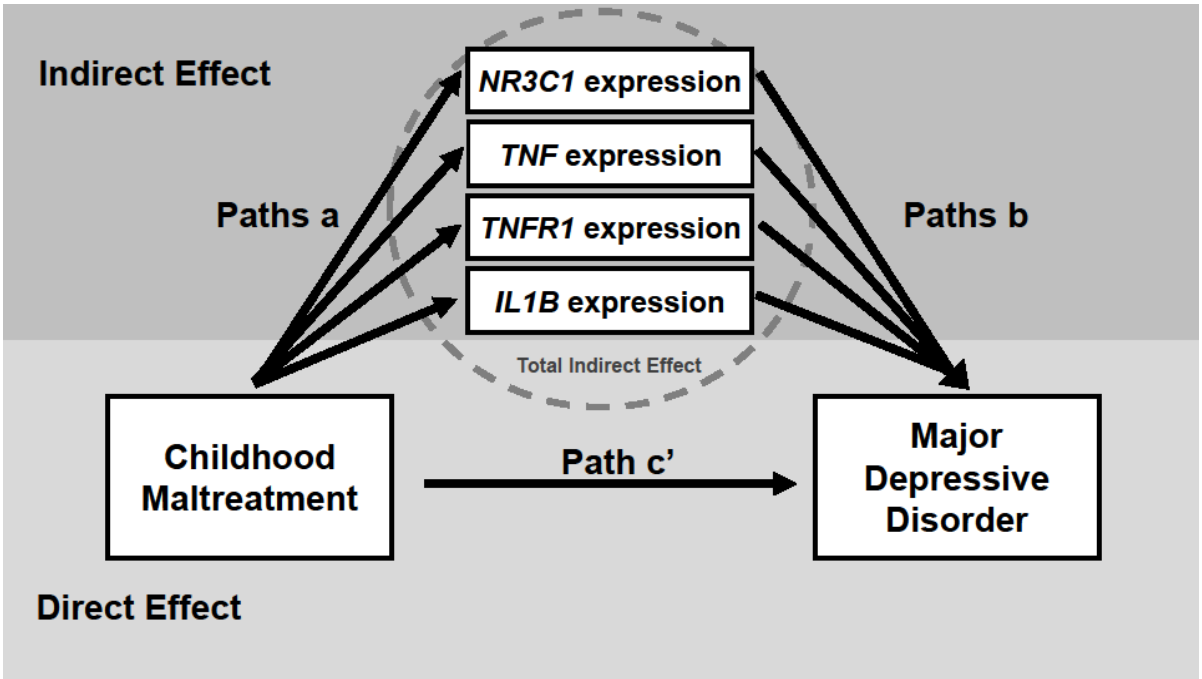

Figure 4.1.1 Representation of the mediation model used to test if childhood maltreatment history could cause MDD directly (direct effect) or indirectly (indirect effect) via gene expression of NR3C1, TNF, TNFR1 and IL1B. The effect of childhood maltreatment history on each gene expression is call path a; the effect of each gene expression on the disorder is call path b; and the effect of childhood maltreatment history on MDD is call path c'. The direct effect is represented by path c' and the indirect effect of each gene is compound by the product of path a and path b. The total indirect effect is the aggregation of the expression of NR3C1, TNF, TNFR1 and IL1B.

4.1.4 Results

We analyzed the mRNA levels of 12 candidate genes in 20 children with MDD, 49 children without MDD but with high levels of depressive symptoms and 61 controls. The characteristics of the participants in this study are given in Table 1. The groups did not differ regarding gender frequencies and mean age. However, we have found differences in site. Children from MDD and DS groups were more frequent in Porto Alegre than Sao Paulo. As expected, both MDD and DS groups showed increased CM

history score when compared to controls. Moreover, both groups showed increased total CBLC score when compared to HC. Regarding comorbidities, 68% of children from MDD group and 59.6% of children from DS group were diagnosed with other psychiatric disorders according to DAWBA. The most frequent diagnoses for both groups were ADHD, ODD and anxiety disorder. Both groups did not differ concerning the frequency of co-morbid psychiatric diagnoses ($p>0.05$ for all the aforementioned diagnoses) (table S4).

Gene expression differences among MDD, DS and HC groups

The Univariate GLM p-values and ΔCrt means of all genes in the groups are reported in Table S5. As gene expression is inversely proportional to ΔCrt values, we have found that NR3C1, TNF, TNFR1 and IL1B showed decreased mRNA levels in MDD group compared to HC (Fold: NR3C1=-1.32; TNF=-1.74; TNFR1=-2.04; IL1B=-1.66) and to DS group (Fold: NR3C1=-1.40; TNF=-1.85; TNFR1=-2.25; IL1B=-1.82) after Bonferroni correction (Figure 2). Moreover, we found a high observed power for the four genes with low to moderate effect size (Table S5). We have not observed significant differences between DS and HC groups. We also have not observed significant differences comparing HC and the co-morbid disorders ($p > 0.05$ for all the aforementioned diagnoses). Comparing only the DEGs between children of MDD group with CBCL DSM-oriented affective score ≥ 10 (subset of MDD group) and controls, the results remain significant (Table S6).

Mediation model with the DEGs

We carried out a parallel multiple mediation model to investigate whether gene expression of NR3C1, TNF, TNFR1 and IL1B (all the four mediators were analyzed conjunctly) could underlie the association between CM history and MDD. We have found that the direct effect of CM history on MDD was not statistically significant. Regarding the indirect effects, we found lack of evidence regarding specific indirect effect for each gene mediating individually the association between CM history and MDD, but we have observed that the total indirect mediation effect (considering the summing of the effects of the 4 genes in the model) was significant (Table 2). As zero is not included in the confidence interval, we could claim an indirect total effect different from zero with 95% confidence. In other words, we have found that there is an indirect effect between CM history and MDD on the gene four expression’s aggregation, but

Table 4.1.1 Demographical and clinical characteristics of the participants.

Variables	MDD	DS	HC	p	p *Post Hoc*		
					MDD *vs.* HC	MDD *vs.* DS	DS *vs.* HC
Gender (males:females)	**7:13**	**26:23**	**36:25**	**0.244**	**--**	**--**	**--**
Site (PA:SP)	**16:4**	**33:16**	**16:45**	**< 0.001**	**--**	**--**	**--**
Age, mean years (SD)	**9.80 (2.07)**	**10.18 (1.74)**	**9.48 (2.18)**	**0.177**	**0.716**	**0.826**	**0.180**
CBCL total, mean score (SD)	**89.80 (40.96)**	**95.96 (17.90)**	**15.77 (10.47)**	**< 0.001**	**< 0.001**	**0.597**	**< 0.001**
CBCL DSM-oriented, mean score (SD)	**10.39 (5.57)**	**12.78 (2.54)**	**0.00 (0.00)**	**< 0.001**	**< 0.001**	**0.004**	**< 0.001**
CM History, mean score (SD)	0.50 (0.51)	0.33 (0.55)	0.01 (0.62)	0.001	0.003	0.517	0.014

The p-values were calculated using chi-squared tests (for gender and site) and one-way analysis of variance with Scheffe post-hoc (for age, CBCL total, CBCL DSM-Oriented and CM history). PA: Porto Alegre; SP: São Paulo; SD: standard deviation; MDD: major depressive disorder group; DS: high levels of depressive symptoms group; HC: healthy controls group; CBCL: Child Behavior Checklist; CM: childhood maltreatment.

not specifically through each mediator. Moreover, we also have generated a bootstrap confidence interval for all possible pairwise comparisons between each specific genes' indirect effects and we found lack of statistical significance among their differences. The β coefficients, R, logit, p-values and 95% confidence intervals of the mediation model are given in Table S7. Extending our second main aim, we also assessed the specificity of the mediation model in terms of outcome measure. Substituting MDD by conduct disorder, we have found that both direct and indirect effects (individually and conjunctly for DEGs) of CM history on CD/ODD were not statistically significant (Table S8).

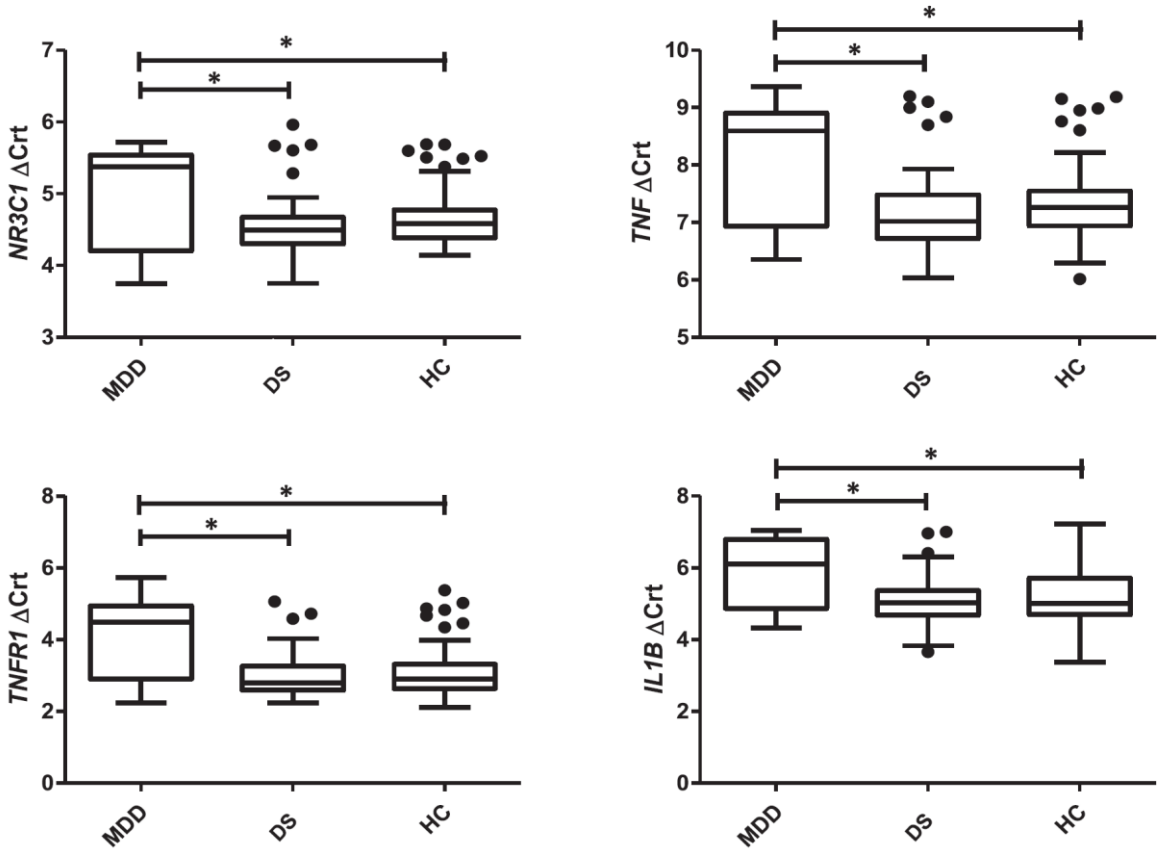

Figure 4.1.2 Error bars graph of ΔCrt values of differentially expressed genes (NR3C1, TNF, TNFR1 and IL1B) among children with major depressive disorder (MDD), children without MDD but with high levels of depressive symptoms (DS) and controls (HC). Error bars represent the standard deviation of ΔCrt values, that is inversely proportional to gene expression.
(*) Significant after Bonferroni correction for multiple comparison (p-values < 0.0042)

Table 4.1.2 Direct and indirect effects of childhood maltreatment history on major depressive disorder (MDD) resulting from the mediation model. The bootstrapped confidence interval was reported on logit scale. Confidence intervals that do not include zero indicate a significant effect at the p < 0.05 significance level. The total indirect effect is the aggregation of the expression of NR3C1, TNF, TNFR1 and IL1B.

		Effect	95% bootstrap CI
Direct Effect		-1.947	-4.493 – 0.599
Indirect Effects	Total	3.587	0.008 – 8.520
	NR3C1	-3.972	-9.790 – 6.421
	TNF	3.255	-3.641 – 9.530
	TNFR1	3.749	-7.039 – 10.057
	IL1B	0.560	-7.217 – 8.141

4.1.5 Discussion

We have found that NR3C1, TNF, TNFR1 and IL1B were downregulated in blood of MDD group compared to DS and HC groups. There were no differences of mRNA levels between DS and HC groups. To our knowledge, only two studies have evaluated mRNA levels of candidate genes in the peripheral blood of children and early adolescents with depression[35, 36], but they investigated the expression of different genes. Moreover, we have found that CM history did not impact MDD directly. In fact, the aggregate expression of the 4 genes (total indirect effect), and not the specific indirect effect of each gene's expression, might underlie the link between CM and MDD. These results suggest that NR3C1, TNF, TNFR1 and IL1B expression might be related with MDD diagnosis according to DSM-IV criteria rated by a clinician and not with subthreshold depressive symptoms. Furthermore, the effect of CM history on MDD could be influenced by DEG expression levels in blood of children and adolescents. Our findings suggest that HPA-axis and inflammation might be critical biological systems involved in early MDD pathophysiology, and illustrate, through the mediation model, how a causal agent (i.e., CM) could transmit its effect on MDD.

The GR is a transcriptional factor that regulates the expression of glucocorticoid-responsive genes and functions in inflammatory responses, cellular proliferation and differentiation.[37] Similarly to our results, lower blood expression levels of NR3C1 were observed in adults with MDD.[11, 38] Moreover, three candidate genes related to the inflammatory system (TNF, TNFR1 and IL1B) were downregulated in the MDD group compared to DS and HC groups. TNF and IL1B encode two multifunctional proinflammatory cytokines that mediate inflammatory response and regulate immune function.[39, 40] TNF-α initiates the majority of its biological activities by binding to TNFR1 receptor, encoded by TNFRSF1A gene, also known as TNFR1.[41] In contrast to our results, increased mRNA levels of these genes, mainly TNF, were reported previously in the peripheral blood of MDD patients.[10, 11] However, a study suggested that findings in adults should not necessarily be extrapolated to children, once cytokine production differs in children as compared to adults.[42] Indeed, adulthood MDD might be affected by confounding factors not identified in childhood MDD, such as lifespan, traumatic events, alcohol consumption and smoking habits.

It is not clear, at the moment, why TNF, TNFR1 and IL1B were downregulated in the MDD group compared to DS and HC groups and further studies are necessary.

However, our results corroborate some findings of plasma concentrations in children and adolescents with MDD. In one study, TNF-α and IL-1β concentrations in children and adolescents with MDD did not differ relative to controls, but TNF-α levels were significantly lower in children with dysthymia than in controls.[43] Another study with suicidal adolescents with MDD found that TNF-α concentration was significantly decreased in plasma when compared to non-suicidal adolescents with MDD.[44] Thereby, the underlying mechanisms of MDD in children and adolescents might differ from those of adulthood MDD.

We also verified if CM history affects MDD through gene expression. It has been well described that CM, such as abuse and neglect, significantly increases the risk of psychopathology, including MDD.[45, 46] However, in our mediation model, we observed that this association was linked by the aggregation of the four parallel mediators (DEGs mRNA levels). As mediation models are causal models and carry with them the usual criteria for making causal claims, we suggest that CM affects the expression in blood of the four DEGs, and this, in turn, affects the risk of developing MDD. However, no significant indirect effect was observed when each gene was considered individually in the model. Moreover, CM history did not affect CD/ODD directly or indirectly, showing the specificity of our model. Considering the complexity of MDD etiology, our findings corroborate the hypothesis of interplay between environmental and genetic factors, because the relationship between CM history and MDD was mediated by the indirect effect of the aggregate expression of DEGs. Nonetheless, we did not know if the indirect effect was conditioned by other factors that could affect MDD, such as gender (moderated moderation model). Although it seems that the genetic susceptibility to MDD might not be shared between gender[47], adding gender in our model could overfit the model given the large number of variables and the small sample size.

It is important to note that gene expression did not differ between DS and HC groups. The DS group comprised children presenting high psychopathology related to affective disorders and diagnosed with psychiatric disorders such as ADHD and anxiety disorder, but not MDD. Psychopathology related to affective problems (measured using CBCL DSM-Oriented scores) was considerably higher in the DS group than in the MDD group, but this does not mean that the DS group children were in a depressive episode at blood collection, because CBCL is not a diagnostic instrument. In this regard, clinician´s rating might capture specific aspects of reported

symptoms, such as level of associated impairment and suffering, as reported by the parent. Moreover, we obtained the same results when we compared controls with children of MDD group with CBCL DSM-oriented scores ≥ 10. Furthermore, because the DS group only included participants with CBCL DSM-Oriented scores of ≥10, this group was expected to present higher psychopathology than the MDD group. Therefore, the high CBCL DSM-oriented score in the DS group could be related to others psychiatric disorders (not MDD), and the lower DEGs mRNA levels in blood of children with MDD compared to DS group might reflect specific aspects related to MDD trait and progression and not to MDD psychopathology.

Our study's strengths are the following: the sample was composed of children and early adolescents with MDD, a group that presents shorter illness exposure relative to adults; this offers the advantage of relatively greater genetic contribution. Furthermore, no participant had used psychiatric medication in the month preceding blood collection. Consequently, our results might be not influenced by antidepressant treatment, a known factor that alters gene expression levels.[48] We investigated mRNA levels in blood, a peripheral tissue that might translate faster into clinical practice. Although the study design was cross-sectional, we used mediation analysis based on assuming a causal relationship in the system, with gene expression located between CM and MDD. Therefore, with the required cautions considered given the nature of our data, the employed statistical method can facilitate understanding of—in an applicable manner—the relationships between the 2 consequent variables (gene expression and MDD) and 2 antecedent variables (CM and gene expression). Lastly, among studies analyzing gene expression in the blood of children and adolescents with depression, our sample of children with MDD is, to our knowledge, the largest ever investigated.

The results of this study should be interpreted in light of some limitations. First, we evaluated the expression of only 12 candidate genes, although a genome-wide approach would more advisable, allowing an exploratory analysis. Second, given that whole blood presents a mixture of various leukocyte subtypes, our expression findings might be partially confounded by distinct leukocyte-subtypes proportions.[49] Third, the blood collection and psychiatric assessment were performed on different days. Fourth, MDD prevalence differed between the 2 study sites, which could be related to existing ethnic, cultural, environmental and socioeconomic differences between them. Indeed, Almeida-Filho et al (1997)[50] showed that MDD prevalence exhibits considerable regional variation in Brazil (from <3% in São Paulo and Brasilia to 10% in Porto

Alegre).[50] To address this issue, we used site as a covariate in all analyses, and the main results remained significant beyond the effect of this confounder. Additionally, one can argue that our multisite and community-based design are strengths, since most of previous studies relied on clinical or referred samples. Fifth, no gender-related differences were found among preschool children, but previous evidence has suggested mood-disorder predominance in females relative to males after puberty.[47] Although we controlled GLM analyses by gender (because our sample included adolescents), our analyses could be further controlled using puberty measures. Sixth, despite the relatively low occurrence of depressive episodes in children[51], our sample size is relatively small, mainly in mediation analysis. Seventh, our analyses could be controlled using sleep and circadian measures, since the expression of the candidates genes may be affect by circadian and sleep variation and there are evidences linking circadian rhythm disorders/sleep disturbances and mood disorders.[52, 53]

In conclusion, we demonstrated that NR3C1, TNF, TNFR1 and IL1B expression levels are related to early stage MDD and that the indirect effect of the aggregate expression of these genes mediates the association between CM history and risk of developing MDD. Our results corroborate the notion that the expression of these genes might underlie the pathophysiology of MDD in children and adolescents. Moreover, we used a causal model that demonstrated that the relationship between CM history and risk of developing MDD was mediated by the aggregation of NR3C1, TNF, TNFR1 and IL1B expression's indirect effects. Further studies are necessary to validate the role of these genes in blood of children and adolescents with MDD.

4.1.6 References

1. Kendler KS, Gatz M, Gardner CO, Pedersen NL. A Swedish national twin study of lifetime major depression. The American journal of psychiatry. 2006;163(1):109-14.

2. Malter Cohen M, Jing D, Yang RR, Tottenham N, Lee FS, Casey BJ. Early-life stress has persistent effects on amygdala function and development in mice and humans. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(45):18274-8.

3. Salum GA, DeSousa DA, Manfro GG, Pan PM, Gadelha A, Brietzke E, et al. Measuring child maltreatment using multi-informant survey data: a higher-order confirmatory factor analysis. Trends Psychiatry Psychother. 2016.

4. Dvir Y, Ford JD, Hill M, Frazier JA. Childhood maltreatment, emotional dysregulation, and psychiatric comorbidities. Harv Rev Psychiatry. 2014;22(3):149-61.

5. Nanni V, Uher R, Danese A. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. The American journal of psychiatry. 2012;169(2):141-51.

6. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends in neurosciences. 2008;31(9):464-8.

7. Pariante CM. Glucocorticoid receptor function in vitro in patients with major depression. Stress. 2004;7(4):209-19.

8. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biological psychiatry. 2001;49(5):391-404.

9. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biological psychiatry. 2009;65(9):732-41.

10. Tsao CW, Lin YS, Chen CC, Bai CH, Wu SR. Cytokines and serotonin transporter in patients with major depression. Progress in neuro-psychopharmacology & biological psychiatry. 2006;30(5):899-905.

11. Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, et al. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. Neuropsychopharmacology. 2013;38(3):377-85.

12. Himmerich H, Fulda S, Linseisen J, Seiler H, Wolfram G, Himmerich S, et al. Depression, comorbidities and the TNF-alpha system. Eur Psychiatry. 2008;23(6):421-9.

13. Lang UE, Borgwardt S. Molecular mechanisms of depression: perspectives on new treatment strategies. Cell Physiol Biochem. 2013;31(6):761-77.

14. Frodl T, O'Keane V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiology of disease. 2013;52:24-37.

15. Zhao YJ, Du MY, Huang XQ, Lui S, Chen ZQ, Liu J, et al. Brain grey matter abnormalities in medication-free patients with major depressive disorder: a meta-analysis. Psychological medicine. 2014;44(14):2927-37.

16. Kendell SF, Krystal JH, Sanacora G. GABA and glutamate systems as therapeutic targets in depression and mood disorders. Expert Opin Ther Targets. 2005;9(1):153-68.

17. Kucukibrahimoglu E, Saygin MZ, Caliskan M, Kaplan OK, Unsal C, Goren MZ. The change in plasma GABA, glutamine and glutamate levels in fluoxetine- or S-citalopram-treated female patients with major depression. Eur J Clin Pharmacol. 2009;65(6):571-7.

18. Kim JS, Schmid-Burgk W, Claus D, Kornhuber HH. Increased serum glutamate in depressed patients. Archiv fur Psychiatrie und Nervenkrankheiten. 1982;232(4):299-304.

19. Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet. 2006;141B(3):261-8.

20. Hepgul N, Cattaneo A, Zunszain PA, Pariante CM. Depression pathogenesis and treatment: what can we learn from blood mRNA expression? BMC medicine. 2013;11:28.

21. Jansen R, Penninx BW, Madar V, Xia K, Milaneschi Y, Hottenga JJ, et al. Gene expression in major depressive disorder. Molecular psychiatry. 2016;21(3):339-47.

22. Flint J, Kendler KS. The genetics of major depression. Neuron. 2014;81(3):484-503.

23. Pietschmann P, Gollob E, Brosch S, Hahn P, Kudlacek S, Willheim M, et al. The effect of age and gender on cytokine production by human peripheral blood mononuclear cells and markers of bone metabolism. Experimental gerontology. 2003;38(10):1119-27.

24. Mitchell RH, Goldstein BI. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. Journal of the American Academy of Child and Adolescent Psychiatry. 2014;53(3):274-96.

25. Salum GA, Gadelha A, Pan PM, Moriyama TS, Graeff-Martins AS, Tamanaha AC, et al. High risk cohort study for psychiatric disorders in childhood: rationale, design, methods and preliminary results. International journal of methods in psychiatric research. 2015;24(1):58-73.

26. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an

integrated assessment of child and adolescent psychopathology. Journal of child psychology and psychiatry, and allied disciplines. 2000;41(5):645-55.

27. Achenbach TM. Manual for the Child Behavior Checklist/4-18 and 1991 Profile. University of Vermont Department of Psychiatry1991.

28. Ebesutani C, Bernstein A, Nakamura BJ, Chorpita BF, Higa-McMillan CK, Weisz JR. Concurrent Validity of the Child Behavior Checklist DSM-Oriented Scales: Correspondence with DSM Diagnoses and Comparison to Syndrome Scales. Journal of psychopathology and behavioral assessment. 2010;32(3):373-84.

29. Amorim P, Lecrubier Y, Weiller E, Hergueta T, Sheehan D. DSM-IH-R Psychotic Disorders: procedural validity of the Mini International Neuropsychiatric Interview (MINI). Concordance and causes for discordance with the CIDI. Eur Psychiatry. 1998;13(1):26-34.

30. Cicchetti D. Defining child maltreatment: The interface between policy and research. Child abuse, child development, and social policy. Norwood, NJ: Ablex; 1993. p. 7-73.

31. Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, et al. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics. 2008;2008:420747.

32. Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004;64(15):5245-50.

33. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behavior research methods, instruments, & computers : a journal of the Psychonomic Society, Inc. 2004;36(4):717-31.

34. Villodas MT, Litrownik AJ, Thompson R, Jones D, Roesch SC, Hussey JM, et al. Developmental transitions in presentations of externalizing problems among boys and girls at risk for child maltreatment. Development and psychopathology. 2015;27(1):205-19.

35. Pandey GN, Dwivedi Y, Rizavi HS, Ren X, Zhang H, Pavuluri MN. Brain-derived neurotrophic factor gene and protein expression in pediatric and adult depressed subjects. Progress in neuro-psychopharmacology & biological psychiatry. 2010;34(4):645-51.

36. Pajer K, Andrus BM, Gardner W, Lourie A, Strange B, Campo J, et al. Discovery of blood transcriptomic markers for depression in animal models and pilot validation in subjects with early-onset major depression. Translational psychiatry. 2012;2:e101.

37. Charmandari E. Primary generalized glucocorticoid resistance and hypersensitivity. Hormone research in paediatrics. 2011;76(3):145-55.

38. Matsubara T, Funato H, Kobayashi A, Nobumoto M, Watanabe Y. Reduced Glucocorticoid Receptor alpha Expression in Mood Disorder Patients and First-Degree Relatives. Biological psychiatry. 2006;59(8):689-95.

39. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature. 1984;312(5996):724-9.

40. March CJ, Mosley B, Larsen A, Cerretti DP, Braedt G, Price V, et al. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature. 1985;315(6021):641-7.

41. Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. Science (New York, NY. 2002;296(5573):1634-5.

42. Lilic D, Cant AJ, Abinun M, Calvert JE, Spickett GP. Cytokine production differs in children and adults. Pediatric research. 1997;42(2):237-40.

43. Brambilla F, Monteleone P, Maj M. Interleukin-1beta and tumor necrosis factor-alpha in children with major depressive disorder or dysthymia. Journal of affective disorders. 2004;78(3):273-7.

44. Gabbay V, Klein RG, Guttman LE, Babb JS, Alonso CM, Nishawala M, et al. A preliminary study of cytokines in suicidal and nonsuicidal adolescents with major depression. Journal of child and adolescent psychopharmacology. 2009;19(4):423-30.

45. Mullen PE, Martin JL, Anderson JC, Romans SE, Herbison GP. The long-term impact of the physical, emotional, and sexual abuse of children: a community study. Child abuse & neglect. 1996;20(1):7-21.

46. McEwen BS. Early life influences on life-long patterns of behavior and health. Mental retardation and developmental disabilities research reviews. 2003;9(3):149-54.

47. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. The American journal of psychiatry. 2000;157(10):1552-62.

48. Ota VK, Noto C, Gadelha A, Santoro ML, Ortiz BB, Andrade EH, et al. Evaluation of neurotransmitter receptor gene expression identifies GABA receptor changes: a follow-up study in antipsychotic-naive patients with first-episode psychosis. Journal of psychiatric research. 2014;56:130-6.

49. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, et al. Individuality and variation in gene expression patterns in human blood. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(4):1896-901.

50. Almeida-Filho N, Mari Jde J, Coutinho E, Franca JF, Fernandes J, Andreoli SB, et al. Brazilian multicentric study of psychiatric morbidity. Methodological features and prevalence estimates. The British journal of psychiatry : the journal of mental science. 1997;171:524-9.

51. Goldman S. Developmental epidemiology of depressive disorders. Child and adolescent psychiatric clinics of North America. 2012;21(2):217-35, vii.

52. Stubbs B, Vancampfort D, Veronese N, Solmi M, Gaughran F, Manu P, et al. The prevalence and predictors of obstructive sleep apnea in major depressive disorder, bipolar disorder and schizophrenia: A systematic review and meta-analysis. Journal of affective disorders. 2016;197:259-67.

53. Melo MC, Abreu RL, Linhares Neto VB, de Bruin PF, de Bruin VM. Chronotype and circadian rhythm in bipolar disorder: A systematic review. Sleep Med Rev. 2016.

4.1.7 Supplemental Information

Study Population

This study is part of a large, community school-based survey that combines standardized evaluation with a neurodevelopmental approach. The High Risk Cohort (HRC) Study for Psychiatric Disorders aims to map neurodevelopmental trajectories in typical development and in common mental illnesses.(1) The baseline assessment was performed in 4 phases, as previously described: 1) screening; 2) household parent interview; 3) child cognitive evaluation (school interview); and 4) neuroimaging and peripheral-biomarker testing. The study population in the screening phase comprised 6–12-year-old students from 22 public schools in Porto Alegre and 35 schools in São Paulo, Brazil. Inclusion criteria were as follows: registered for school by a biological parent capable of providing consent and information regarding the child's behavior; age between 6 and 12 years; and enrolled in the same school during the year. For screening, 9,937 informant interviews (88% with biological mothers) on the Family History Survey (FHS) were conducted.(2) From this pool, we selected 2 subgroups: a community random group and a high-risk of psychiatric disorder stratum. From the FHS, we extracted an index of family load that expressed the percentage of family members who screened positive for the psychiatric disorders evaluated. The high-risk group was determined based on this index.

From 1,315 children selected for the random stratum, 958 (73%) completed the household parent interview phase. From the 2,050 children selected for the high-risk stratum, 1,553 (76%) participated in the study. The entire sample composed of the random and high-risk groups included 2,511 participants. The structured diagnostic interview Development and Well-Being Assessment (DAWBA) was used to evaluate psychiatric diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV).(3) The biological mother was the respondent in 94.5% of the interviews. DAWBA rating procedures followed the recommendations of the instrument (further information available at www.dawbainfo.com). Trained lay interviewers asked structured questions regarding common psychiatric symptoms in children and adolescents that are closely related to DSM diagnostic criteria for several prevalent disorders in this age group. The interviewers also asked for detailed information on each positive rated symptom by using open-ended questions, and thus evaluated each symptom and its consequent impairment. All interviews were then

evaluated, using a computer platform, by 9 trained psychiatrist raters. Raters received continuous supervision after an initial training provided by an experienced child psychiatrist, who was also highly experienced in using DAWBA rating procedures. Inter-rater agreement was evaluated for a subset of 200 interviews, and for emotional disorders, the results were good to excellent (k = 0.85; agreement 95.48%, expected 70.65%, z = 11.98, $p < 0.001$).

From the total cohort of 2,512 participants, 1,004 children were invited to participate in neuroimaging and peripheral-biomarker testing; 751 children (and their parents/guardians) accepted the invitation to participate in MRI scanning, and 625 agreed to blood-sample collection. We collected blood in one EDTA tube (Becton Dickinson (BD), Franklin Lakes, NJ) for DNA analysis; one PAXgene® RNA tube (PreAnalytix, Hombrechtikon, Switzerland) for RNA analysis; and one Gel SST II Advance tube (BD) for protein analysis. On the day of blood collection, the parents/caregivers also completed the Child Behavior Checklist (CBCL), a parent-report questionnaire based on which each child was rated for various behavioral and emotional problems.[4]

Blood-Sample Preparation and Gene-Expression Analysis

A total of 5 mL of whole blood was collected in PAXgene® RNA tubes and, subsequently, RNA was isolated using a PAXgene® Blood RNA kit (Qiagen, Stockach, Germany), according to the manufacturer's instructions. RNA integrity was verified using electrophoresis on 1.0% agarose gels, and the quality and quantity of the RNA samples were determined using a NanoDrop® ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). cDNA was synthetized using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Foster City, CA), with a standard RNA input of 400 ng. Gene-expression analysis was performed using TaqMan® Low Density Array (TLDA) microfluidic cards and ViiA™ 7 Real-Time PCR System (both from Life Technologies), as per manufacturer instructions. Probes and primers of 13 target genes, 2 housekeeping genes (*ACTB*, *GAPDH*), and one PCR positive control (18S), all in triplicates, were preloaded in the 384 wells of each TLDA card. One target gene, *CACNA1C*, was not amplified and was therefore excluded from all analyses.

Gene expression was quantified using the relative threshold (Crt) method with the geometric mean (GM) between *GAPDH* and *ACTB* gene expression as endogenous control. ΔCrt values ($\Delta Crt = Crt_{target\ gene} - Crt_{GM\ GAPDH/ACTB}$) were calculated for each sample. We have tested *ACTB* and *GAPDH* genes in blood samples and

found a strong correlation between them and no association with MDD diagnosis and psychopathology symptoms. Moreover, we used the NormFinder algorithm for identifying the optimal normalization gene among our set of candidate genes and, among all genes, *ACTB* and *GAPDH* presented the smallest stability values.[5]

Statistical analysis

We performed one-way ANOVA to verify if there were differences among the three groups (MDD, DS and HC) regarding age, CBCL total, CBCL DSM-oriented and CM history scores. Gender and site (Porto Alegre or Sao Paulo) effects were tested using the chi-squared test. We also used chi-squared test to verify if there were differences between MDD and DS groups regarding the presence of any psychiatric comorbidities.

Table 4.1.S1 Information about history of childhood maltreatment (CM) considering the child report, the parents report and both reports. All these variables are latent variables generated by confirmatory factor analysis. For this study, we only used the CM considering both child and parent reports.

Variables		CM Child Report Mean (SD)	CM Parent report Mean (SD)	CM Child + Parent Report Mean (SD)
Groups	MDD	0.471 (0.76)	0.901 (0.96)	0.503 (0.49)
	DS	0.408 (0.77)	0.565 (1.02)	0.343 (0.54)
	HC	0.090 (0.79)	-0.023 (1.07)	0.013 (0.61)
Gender	Male	0.282 (0.83)	0.367 (1.12)	0.227 (0.63)
	Female	0.269 (0.75)	0.353 (1.07)	0.218 (0.56)
ODD	Yes	0.626 (0.84)	0.903 (0.90)	0.542 (0.47)
	No	0.246 (0.78)	0.314 (1.09)	0.196 (0.60)
CD	Yes	0.622 (0.86)	0.322 (1.11)	0.196 (0.60)
	No	0.235 (0.78)	0.322 (1.11)	0.444 (0.50)

For more information, see (6). CM: childhood maltreatment; SD: standard deviation; MDD: major depressive disorder group; DS: high levels of depressive symptoms group; HC: healthy controls group; ODD: oppositional defiant disorder; CD: conduct disorder.

Table 4.1.S2 Information about the candidate genes selected for this study (biological system, official full name, function and citation of previous studies).

Biological System	Gene Symbol	Official Full Name	Function	Citation
Neurodevelopment	DISC1	disrupted in schizophrenia 1	Protein involved in neurite outgrowth and cortical development	Insoluble DISC1proteins (which a loss-of-function phenotype could be demonstrated) were reported in the postmortem brains of sporadically collected patients with schizophrenia, MDD and bipolar disorder (7)
Neurodevelopment	PDE4B	phosphodiesterase 4B, cAMP-specific	Cyclic nucleotide phosphodiesterase that regulates the cellular concentrations of cyclic nucleotides and thereby play a role in signal transduction.	Increased mRNA levels in blood of patients with MDD and decreased mRNA levels after antidepressant treatment (8)
Neurodevelopment	QKI	Quaking homologue, KH domain containing, RNA binding	RNA-binding protein that regulates pre-mRNA splicing, export of mRNAs from the nucleus, protein translation, and mRNA stability	Decreased mRNA levels in 11 in cortical and subcortical brain regions of suicide victims with MDD diagnosis (9)
Neurodevelopment	CACNA1C(*)	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	Alpha-1 subunit of a voltage-dependent calcium channel that binds to and is inhibited by dihydropyridine.	CACNA1C has been associated with depressive psychopathology and methylation changes of CACNA1C have been associated with early-life stress, a risk factor for MDD (10-12)
Neurotransmission	SLC1A4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	A sodium-dependent neutral amino acid transporter for alanine, serine, cysteine, and threonine	Decreased immunoreactivity in neurons of patients with MDD (13)

Neurotransmission	GLUL	glutamate-ammonia ligase	Glutamine synthetase protein that catalyzes the synthesis of glutamine from glutamate and ammonia in an ATP-dependent reaction.	Decreased mRNA levels in the locus coeruleus tissue from postmortem brains of patients with MDD (14)
Neurotransmission	COMT	catechol-O-methyltransferase	It catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines	COMT has been associated with MDD with possible distinct effects in gender and different ethnic populations (15, 16)
HPA axis	NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	Glucocorticoid receptor, which can function both as a transcription factor and as a regulator of other transcription factors	Decreased mRNA levels in blood of patients with MDD compared with controls and increased mRNA levels after antidepressant treatment (17, 18)
HPA axis	FKBP5	FK506 binding protein 5	a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes	Increased mRNA levels in blood of patients with MDD compared with controls and decreased mRNA levels after antidepressant treatment (17)
Inflammation	TNF	tumor necrosis factor	A multifunctional proinflammatory cytokine involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation and apoptosis	Increased mRNA levels in blood of patients with MDD compared with controls and prediction of antidepressant response (17, 19)
Inflammation	TNFR1 (Official Symbol TNFRSF1A)	tumor necrosis factor receptor superfamily, member 1A	A member of the TNF receptor superfamily that is found in membrane-bound and soluble forms that interact with its ligand, tumor	Increased soluble plasma TNFR1 levels in patients with MDD compared with controls and increased mRNA levels in blood of

			necrosis factor alpha	patients with recurrent MDD compared with controls (20, 21)
Inflammation	TNFR2 (Official Symbol TNFRSF1B)	tumor necrosis factor receptor superfamily, member 1B	A member of the TNF receptor superfamily with the function in TNF-receptor signaling unknown	Increased soluble plasma TNFR2 levels in patients with MDD compared with controls and decreased mRNA levels in the Brodmann's area 46 in patients with MDD (20, 22)
Inflammation	IL1B	interleukin 1, beta	A member of the interleukin 1 cytokine family that is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation and differentiation	Increased mRNA levels in patients with MDD compared with controls and prediction of antidepressant response (17, 19)

(*) CACNA1C was not amplified in the TaqMan® Low Density Array (TLDA) microfluidic cards and was therefore excluded from all analyses. MDD: major depressive disorder.

Table 4.1.S3 Assays used in the real time PCR, identification of the transcripts and exons that are recognized in each reaction.

Gene symbol	Assay ID	NCBI Reference Sequence (Refseq)	Exon boundary
NR3C1	Hs00353740_m1	NM_000176.2	4 – 5
		NM_001018074.1	4 – 5
		NM_001018075.1	4 – 5
		NM_001018076.1	4 – 5
		NM_001018077.1	4 – 5
		NM_001020825.1	4 – 5
		NM_001024094.1	4 – 5
		NM_001204258.1	4 – 5
		NM_001204259.1	4 – 5
		NM_001204260.1	4 – 5
		NM_001204261.1	4 – 5
		NM_001204262.1	4 – 5
		NM_001204263.1	4 – 5
		NM_001204264.1	4 – 5
		NM_001204265.1	4 - 5
FKBP5	Hs01561006_m1	NM_001145775.1	6 – 7
		NM_001145776.1	5 – 6
		NM_001145777.1	5 – 6
		NM_004117.3	5 - 6
TNF	Hs01113624_g1	NM_000594.3	2 - 3
TNFR1	Hs00533568_g1	NM_001065.3	3 - 4
TNFR2	Hs00961749_m1	NM_001066.2	2 - 3
IL1B	Hs01555410_m1	NM_000576.2	3 - 4
DISC1	Hs00257791_s1	AK023443.1*	1 - 1
PDE4B	Hs00963643_m1	NM_001037339.1	3 – 4
		NM_001037340.1	8 – 9
		NM_001037341.1	10 – 11
		NM_002600.3	10 - 11
QKI	Hs00916681_m1	NM_006775.2	7 - 8
SLC1A4	Hs00161719_m1	NM_001193493.1	6 – 7
		NM_003038.4	7 - 8
GLUL	Hs00365928_g1	NM_001033044.2	2 – 3
		NM_001033056.2	2 – 3
		NM_002065.5	3 – 4
COMT	Hs02511558_s1	NM_000754.3	6 – 6
		NM_001135161.1	6 – 6
		NM_001135162.1	6 – 6
		NM_007310.2	4 – 4
CACNA1A	Hs01579431_m1	NM_000068.3	41 – 42
		NM_001127221.1	40 – 41
		NM_001127222.1	40 – 41
		NM_001174080.1	41 – 42
		NM_023035.2	41 - 42
GAPDH	Hs02786624_g1	NM_001256799.1	7 – 7
		NM_002046.4	8 - 8
ACTB	Hs01060665_g1	NM_001101.3	2 - 3
18S	Hs99999901_s1	X03205.1*	1 - 1

(*) Identification of the transcripts based only on GenBank archival sequence database.

Table 4.1.S4 The prevalence of the most prevalent co-morbid disorders (according to DAWBA) in children with major depressive disorder (MDD) and children without MDD but with high levels of depressive symptoms (DS).

Co-morbidity		MDD N = 20 subjects	DS N = 49 Subjects	p-Value
ADHD	Yes	3 (15%)	10 (20.4%)	0.739
	No	17 (85%)	39 (79.6%)	
ODD	Yes	5 (25%)	5 (10.2%)	0.579
	No	15 (75%)	44 (89.8%)	
CD	Yes	1 (5%)	0 (0%)	0.081
	No	19 (95%)	49 (100%)	
Anxiety	Yes	7 (35%)	20 (40.8%)	0.624
	No	13 (65%)	29 (59.2%)	

The p-values were calculated using chi-squared tests. ADHD: Attention deficit hyperactivity disorder; ODD: oppositional defiant disorder; CD: conduct disorder; MDD: major depressive disorder group; DS: high levels of depressive symptoms group.

Table 4.1.S5 ΔC_{rt} values of genes belonging to HPA axis (*NR3C1* and *FKBP5*), inflammation (*TNF*, *TNFR1*, *TNFR2* and *IL1B*), neurodevelopment (*DISC1*, *PDE4B* and *QKI*) and neurotransmission (*SLC1A4*, *GLUL* and *COMT*) in children with major depressive disorder (MDD), children without MDD but with high levels of depressive symptoms (DS) and controls (HC).

Genes	MDD ΔCrt mean (SD)	DS ΔCrt mean (SD)	HC ΔCrt mean (SD)	F	p-Value	Partial Eta Square	Observed power	p *Post Hoc*		
								MDD *vs.* HC	MDD *vs.* DS	DS *vs.* HC
NR3C1	5.06 (0.66)	4.57 (0.45)	4.66 (0.40)	7.644	**0.001**	**0.116**	**0.943**	**0.013**	**0.001**	**0.509**
FKBP5	5.01 (0.60)	4.95 (0.41)	4.92 (0.41)	0.345	0.709	0.006	0.104	0.790	0.926	0.932
TNF	8.13 (1.01)	7.24 (0.72)	7.33 (0.69)	8.119	**0.001**	**0.124**	**0.955**	**< 0.001**	**< 0.001**	**0.789**
TNFR1	4.14 (1.11)	2.97 (0.63)	3.11 (0.73)	12.924	**< 0.001**	**0.186**	**0.997**	**< 0.001**	**< 0.001**	**0.615**
TNFR2	1.83 (0.24)	1.94 (0.27)	2.01 (0.31)	2.486	0.088	0.042	0.490	0.069	0.357	0.512
IL1B	5.97 (0.94)	5.11 (0.68)	5.24 (0.81)	8.720	**< 0.001**	**0.130**	**0.967**	**0.001**	**< 0.001**	**0.692**
DISC1	9.38 (1.41)	8.18 (1.06)	8.47 (1.20)	5.051	0.008	0.105	0.805	0.057	0.011	0.523
PDE4B	5.65 (0.28)	6.03 (0.39)	6.07 (0.42)	3.065	0.050	0.050	0.582	< 0.001	0.002	0.913
QKI	3.64 (0.24)	3.74 (0.22)	3.77 (0.25)	0.449	0.640	0.008	0.122	0.135	0.315	0.819
SLC1A4	8.02 (0.55)	7.92 (0.54)	7.77 (0.55)	2.589	0.080	0.047	0.506	0.224	0.792	0.376
GLUL	2.11 (0.56)	1.57 (0.43)	1.54 (0.44)	5.385	0.006	0.085	0.835	< 0.001	0.003	0.734
COMT	5.99 (0.58)	5.63 (0.45)	5.74 (0.39)	3.693	0.028	0.059	0.668	0.105	0.010	0.370

The p-values were calculated using Univariate General Linear Model. P-values < 0.0042 were considered to be significant after a Bonferroni correction for multiple comparisons (in bold). As post-hoc test, we used Scheffe method. Partial Eta Square values express the effect size which 0.16 - 0.4 are seen as moderate values. SD: Standard deviation; MDD: major depressive disorder group; DS: high levels of depressive symptoms group; HC: healthy controls group.

Table 4.1.S6 ΔCrt values of differentially expressed genes (*NR3C1*, *TNF*, *TNFR1* and *IL1B*) in children of MDD group with CBCL DSM-oriented affective score≥10 (MDD subset) and healthy controls (HC).

Genes	MDD Subset ΔCrt mean (SD) N = 10 subjects	HC ΔCrt mean (SD) N = 61 subjects	F	*p*-Value	Partial Eta Square	Observed power
NR3C1	4.87 (0.68)	4.66 (0.40)	5.024	**0.029**	**0.074**	**0.598**
TNF	8.08 (0.97)	7.33 (0.69)	9.765	**0.003**	**0.136**	**0.868**
TNFR1	4.02 (1.19)	3.11 (0.73)	13.655	**< 0.001**	**0.180**	**0.953**
IL1B	5.88 (0.88)	5.24 (0.81)	6.150	**0.016**	**0.089**	**0.685**

The p-values were calculated using Univariate General Linear Model. P-values < 0.05 were considered to be significant (in bold). Partial Eta Square values express the effect size which 0.16 - 0.4 are seen as moderate values. SD: Standard deviation; MDD: major depressive disorder group; HC: healthy controls group.

Table 4.1.S7 The β coefficients, R, logit, p-values and 95% confidence intervals (CI) of the mediation model with history of childhood maltreatment (CM) as predictor, major depressive disorder (MDD) as outcome and ∆Crt values (which is inversely correlated to gene expression) as mediator, controlling for site.

	Logit		p- Value	95%CI
Direct Effect - Path c' History of CM → MDD	-1.947		0.134	-4.493 – 0.599
	Gene	**R-sq**	**β**	**p- Value**	**95%CI**
Path a History of CM→ Gene expression	*NR3C1*	0.352	0.523	**< 0.001**	**0.355 – 0.691**
	TNF	0.339	0.867	**< 0.001**	**0.580 – 1.153**
	TNFR1	0.385	1.027	**< 0.001**	**0.718 – 1.337**
	IL1B	0.283	0.823	**< 0.001**	**0.513 – 1.134**
	Gene	**Logit**		**p- Value**	**95%CI**
Path b Gene expression→ MDD	*NR3C1*	-7.599		**0.023**	**-14.154 - -1.043**
	TNF	3.756		**0.023**	**0.530 – 6.981**
	TNFR1	3.649		**0.034**	**0.284 – 7.013**
	IL1B	0.676		0.616	-1.962 – 3.312

The values in the table were calculated using the PROCESS macro for SPSS [23]. P-values < 0.05 were considered to be significant (in bold). Direct effect of history of CM on MDD was estimated using the bootstrap bias corrected method that generates 95% confidence intervals (CIs). CIs that do not include zero indicate a statistically significant indirect effect. We used 10,000 bootstrap samples for the analyses. CM: childhood maltreatment; MDD: Major Depressive Disorder; CI: confidence intervals.

Table 4.1.S8 Direct and indirect effects of history of childhood maltreatment on conduct disorder (CD) or oppositional defiant disorder (ODD) resulting from the mediation model.

Outcome			Effect	95% bootstrap CI
CD/ODD (DAWBA criteria)	Direct Effect		0.628	-1.523 – 2.779
	Indirect Effects	Total	0.219	-5.504 – 5.587
		NR3C1	-1.621	-7.510 – 2.983
		TNF	1.280	-5.129 – 5.698
		TNFR1	0.371	-12.475 – 9.567
		IL1B	0.189	-7.105 – 10.160

The direct and indirect effects were calculated using the PROCESS macro for SPSS (Preacher and Hayes, 2004). The effects were estimated using the bootstrap bias corrected method that generates 95% confidence intervals (CIs). The bootstrapped CIs were reported on logit scale. CIs that do not include zero indicate a significant effect at the $p<0.05$ significance level. We used 10,000 bootstrap samples for the analyses. The total indirect effect is the aggregation of the expression of *NR3C1*, *TNF*, *TNFR1* and *IL1B*. CI: confidence intervals. CD: conduct disorder; ODD: oppositional defiant disorder.

4.1.8 Supplemental Information – References

1. Salum GA, Gadelha A, Pan PM, Moriyama TS, Graeff-Martins AS, Tamanaha AC, et al. High risk cohort study for psychiatric disorders in childhood: rationale, design, methods and preliminary results. International journal of methods in psychiatric research. 2015;24(1):58-73.

2. Weissman MM, Wickramaratne P, Adams P, Wolk S, Verdeli H, Olfson M. Brief screening for family psychiatric history: the family history screen. Archives of general psychiatry. 2000;57(7):675-82.

3. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. Journal of child psychology and psychiatry, and allied disciplines. 2000;41(5):645-55.

4. Achenbach TM. Manual for the Child Behavior Checklist/4-18 and 1991 Profile. University of Vermont Department of Psychiatry1991.

5. Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004;64(15):5245-50.

6. Salum GA, DeSousa DA, Manfro GG, Pan PM, Gadelha A, Brietzke E, et al. Measuring child maltreatment using multi-informant survey data: a higher-order confirmatory factor analysis. Trends Psychiatry Psychother. 2016.

7. Goldman S. Developmental epidemiology of depressive disorders. Child and adolescent psychiatric clinics of North America. 2012;21(2):217-35, vii.

8. Numata S, Iga J, Nakataki M, Tayoshi S, Taniguchi K, Sumitani S, et al. Gene expression and association analyses of the phosphodiesterase 4B (PDE4B) gene in major depressive disorder in the Japanese population. Am J Med Genet B Neuropsychiatr Genet. 2009;150B(4):527-34.

9. Klempan TA, Ernst C, Deleva V, Labonte B, Turecki G. Characterization of QKI gene expression, genetics, and epigenetics in suicide victims with major depressive disorder. Biological psychiatry. 2009;66(9):824-31.

10. Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, et al. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. Molecular psychiatry. 2012;17(1):36-48.

11. Oh JE, Chambwe N, Klein S, Gal J, Andrews S, Gleason G, et al. Differential gene body methylation and reduced expression of cell adhesion and neurotransmitter receptor genes in adverse maternal environment. Translational psychiatry. 2013;3:e218.

12. Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. Experimental neurology. 2012;233(1):102-11.

13. Weis S, Llenos IC, Dulay JR, Verma N, Sabunciyan S, Yolken RH. Changes in region- and cell type-specific expression patterns of neutral amino acid transporter 1 (ASCT-1) in the anterior cingulate cortex and hippocampus in schizophrenia, bipolar disorder and major depression. J Neural Transm (Vienna). 2007;114(2):261-71.

14. Kim JS, Schmid-Burgk W, Claus D, Kornhuber HH. Increased serum glutamate in depressed patients. Archiv fur Psychiatrie und Nervenkrankheiten. 1982;232(4):299-304.

15. Wang M, Ma Y, Yuan W, Su K, Li MD. Meta-Analysis of the COMT Val158Met Polymorphism in Major Depressive Disorder: Effect of Ethnicity. J Neuroimmune Pharmacol. 2016;11(3):434-45.

16. Klein M, Schmoeger M, Kasper S, Schosser A. Meta-analysis of the COMT Val158Met polymorphism in major depressive disorder: the role of gender. World J Biol Psychiatry. 2016;17(2):147-58.

17. Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, et al. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. Neuropsychopharmacology. 2013;38(3):377-85.

18. Matsubara T, Funato H, Kobayashi A, Nobumoto M, Watanabe Y. Reduced Glucocorticoid Receptor alpha Expression in Mood Disorder Patients and First-Degree Relatives. Biological psychiatry. 2006;59(8):689-95.

19. Tsao CW, Lin YS, Chen CC, Bai CH, Wu SR. Cytokines and serotonin transporter in patients with major depression. Progress in neuro-psychopharmacology & biological psychiatry. 2006;30(5):899-905.

20. Grassi-Oliveira R, Brietzke E, Pezzi JC, Lopes RP, Teixeira AL, Bauer ME. Increased soluble tumor necrosis factor-alpha receptors in patients with major depressive disorder. Psychiatry Clin Neurosci. 2009;63(2):202-8.

21. Nanni V, Uher R, Danese A. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. The American journal of psychiatry. 2012;169(2):141-51.

22. Lee PR, Brady D, Koenig JI. Corticosterone alters N-methyl-D-aspartate receptor subunit mRNA expression before puberty. Brain Res Mol Brain Res. 2003;115(1):55-62.

23. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behavior research methods, instruments, & computers : a journal of the Psychonomic Society, Inc. 2004;36(4):717-31.

4.2 Study 2: Ventral striatum functional connectivity as a predictor of adolescent depressive disorder in a longitudinal community-based sample

This paper was accepted for publication in The American Journal of Psychiatry.
Impact Factor - 14.18 – 2016 Journal Citation Reports.(69)
ISSN - 0002-953X- Qualis A1, MEDICINA II (2013-2016).

4.2.1 Authors and Abstract

Pedro Mario Pan[1,2], M.D., João Sato[1,3], Ph.D., Giovanni Salum[4], M.D., Ph.D., Luis A. Rohde[4], M.D., Ph.D., Ary Gadelha[1], M.D., Ph.D., Andre Zugman[1], M.D., Ph.D., Jair Mari[1], M.D., Ph.D., Andrea Jackowski[1], M.D., Ph.D., Felipe Picon[4], M.D., Eurípedes C. Miguel[5], M.D., Ph.D., Daniel S. Pine[6], M.D., Ellen Leibenluft[7], M.D., Rodrigo A. Bressan[1,8], M.D., Ph.D., Argyris Stringaris[2], M.D., Ph.D.

[1]Deparment of Psychiatry, Laboratório Interdisciplinar de Neurociências Clínicas (LiNC), Universidade Federal de São Paulo, São Paulo, Brazil.

[2] Mood Brain & Development Unit, Emotion and Development Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA.

[3]Mathematics & Statistics Institute, Universidade Federal do ABC, Santo André, Brazil

[4]Department of Psychiatry, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

[5]Department & Institute of Psychiatry, Universidade de São Paulo, , São Paulo, Brazil

[6]Section on Development and Affective Neuroscience (SDAN), Emotion and Development Branch, National Institute of Mental Health, Bethesda, MD, USA.

[7] Section on Mood Dysregulation and Neuroscience (SMDN), Emotion and Development Branch National Institute of Mental Health, Bethesda, MD, USA.

[8]Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London.

Objective: Prior studies implicate aberrant reward processing in the pathogenesis of adolescent depression. However, no previous study has used functional connectivity within a distributed reward network, assessed using resting-state functional magnetic resonance imaging (r-sfMRI), to predict the onset of depression in adolescents. This study uses reward-network based functional

connectivity at baseline to predict depressive disorder at follow up in a community sample of adolescents.

Method: Six hundred thirty seven 6-12 years-olds underwent r-sfMRI. Discovery and replication analyses tested the intrinsic functional connectivity (iFC) among nodes of a putative reward network. Logistic regression tested whether striatal node strength, a measure of reward-related iFC, predicted onset of a depressive disorder at 3-years follow-up. Further analyses investigated the specificity of this prediction.

Results: Increased left ventral striatum node strength predicted increased risk for future depressive disorder (OR 1.54, 95%CI=1.09-2.18, p=.03), even after excluding subjects with depressive disorders at baseline (OR 1.52, 95%CI 1.05-2.20, p=.03). Among 11 reward-network nodes, only the left ventral striatum significantly predicted depression. Striatal node strength did not predict other common adolescent psychopathology, such as anxiety, ADHD and substance use.

Conclusions: Aberrant ventral striatum functional connectivity specifically predicts future risk for depressive disorder. This finding further emphasizes the need to understand how brain reward networks contribute to youth depression.

4.2.2 Introduction

Major Depressive Disorder (MDD), a leading cause of disease burden[1], commonly begins in adolescence.[2] Expanding knowledge on reward-system function in depression could inform attempts to identify at-risk adolescents. Here, we use a longitudinal design in a community-based sample to test the hypothesis that aberrant intrinsic reward-system connectivity in early adolescence predicts risk for depressive disorder three years later.

The incidence of depression markedly rises in adolescence[2], potentially due to maturing reward-system function.[3] Most evidence linking aberrant reward processing to adolescent depression derives from task-based functional magnetic resonance imaging (fMRI) studies targeting reward-related areas, such as the striatum.[4, 5] However, few studies adopt network-based approaches and most are cross-sectional.[5] Given the distributed nature of neural perturbations in depression, work is needed applying network-based approaches to intrinsic functional connectivity (iFC) data.[6-8] Such work quantifies the degree to which brain "nodes"[9] facilitate signal integration among network components. Applying this approach to longitudinal data could support inferences about causality.[5] While iFC studies implicate reward-network function in depression[10, 11], most studies examine small, clinically-referred, samples of adults on medication. Notably, there is a particular need for longitudinal studies of iFC in adolescent depression, to extend promising cross-sectional results.[12]

We use a longitudinal design to link reward-network iFC to later risk for a depressive disorder in a community-based adolescent sample. Based on previous findings, we hypothesize that aberrant ventral striatal iFC in early adolescence will increase risk for future depressive disorder at three-year follow-up.[4] Prior work suggests that such aberrancies reflect perturbed striatal integration of coalescing signals from a distributed reward network, encompassing the ventral tegmental area, anterior cingulate (ACC) and ventromedial pre-frontal cortex (PFC).[13-15] We quantify this integrative function through a measure of ventral striatum "node strength" (i.e. *degree centrality*), assessed as the region's weighted sum of connection with other reward-network regions. We stringently test this hypothesis by probing the existence of a putative reward network[10] in separate discovery and replication samples, both assessed with relatively conservative statistical thresholds. We then assess specificity by a) evaluating other reward-system-related brain areas in the prediction of

depression and by b) testing striatal node strength as a predictor of other psychiatric outcomes, including anxiety disorders, ADHD, and substance abuse.

4.2.3 Methods

Study Design

Baseline

This study is part of an ongoing cohort study, the High Risk Cohort (HRC). All parents signed informed consent and children provided verbal assent. Ethics committees of all Universities involved in the cohort approved the project. For a detailed description of HRC sampling, see Salum 2015 et al.[16] Fifty seven schools from two Brazilian cities, São Paulo and Porto Alegre, participated. In Brazil, parents are required to register their children in a local school. On school registry day, we invited biological parents of 6-12 year-old children at these schools to participate in the study. Biological parents (mother, 87.3%) answered the Family History Screening (FHS)[17] for 8012 families, representing 9937 children. Only biological parents were eligible. From this pool, we created the HRC by combining two strata (n=2511). The first stratum included a sample of randomly selected subjects, “the random group” (n=958). The second stratum, “the high risk group” (n=1553), included youth at risk for psychopathology, selected using a validated prioritization algorithm [16]. Only one child per family was included. The baseline evaluation included a household lay interview with a biological parent (mother, 94.5%), including extensive risk factor evaluation and structured psychiatric interview using the Developmental and Well-Being Assessment (DAWBA), Brazilian Portuguese version.[18, 19]

Follow Up

Three years later, we contacted parents to participate in the HRC follow-up. The first follow-up evaluation included a household visit by a lay interviewer, who interviewed the parents or main caregivers of study subjects. In a second household visit, certified psychologists interviewed the adolescent with the self-reported version of the DAWBA. In 10.2% (n=255) of cases, we were unable to contact any family member using all available information. Strategies to contact these individuals included phoning family members, calling at several different times of day, searching in school registries, attempting contact by mail, and visiting the address where the baseline evaluation occurred. Another 9.8% (n=246) of families refused to participate in the follow up evaluation. The remaining sample consisted of 2010 subjects, comprising

80.05% of the baseline sample. Higher maternal education (chi-square 14.07, p<.001) and socioeconomic status (chi-square 6.24, p<.05), living in Porto Alegre City (chi-square 4.57, p<.05), and having a child who met criteria for an anxiety disorder at baseline (chi-square 9.754 p<.01) increased the chance of successful follow-up.

Measures

Psychopathology – DAWBA

Data on psychopathology were only obtained from biological parents at baseline. Data suggest that youth report before age 11 is relatively unreliable.[20] Hence, we collected only parent reported symptoms of depression in the child at baseline[18]; for the follow-up, we used both parental and adolescents´ self-report. At this follow-up wave, trained psychiatrist raters evaluated parent- and self-reported information using a digital platform (*youthinmind.com*), which integrates verbatim responses to open-ended questions, thus supplementing information from the structured questions. Inconclusive cases were discussed in research group meetings with senior psychiatrists. Raters were blinded for study site at both time points. At follow-up, they were blinded for baseline psychiatric disorders, but were allowed to integrate parent- and self-report sources of information to arrive at a clinical diagnosis.

“Depressive Disorder

We computed the depressive disorder category by merging DAWBA clinical diagnoses of MDD with the Other Depression category; the latter encompasses DSM´s Other Specified Depressive Disorder and Unspecified Depressive Disorder. Briefly, Other Depression included subjects who met the impairment criterion for major depressive disorder (MDD), but failed to meet specific symptomatic or duration criteria, as assessed by the clinician rating. We also used the “loss of interest” question from DAWBA´s depression section to evaluate anhedonia at baseline. We specifically investigate anhedonia given evidence about its association with reward aberrations in depressive disorder.

Substance Use

We investigated substance use by parent- and self-report, merging any lifetime use of alcohol, tobacco, or any drug into a dichotomized variable, called “any substance use” (ASU).

Use of Medication

We excluded subjects whose parents reported regular use of a psychotropic medication within the 30 days before the MRI scan (n=18). Among those excluded

subjects, eight were taking antidepressants but did not meet criteria for a depressive disorder.

Neuroimaging

Data acquisition

The HRC study's goal was to perform MRI scans at a subsample of the baseline. Subjects who completed household and school evaluation were eligible to participate, following the same procedure of the screening phase. From the pool of 2511 subjects, MRI's were successfully acquired from 741 subjects (see Supplemental Material for full description of procedures). We used 1.5 T MRI systems (GE Signa HDX and GE Signa HD—G.E., USA) at two sites, running identical imaging protocols. fMRI parameters were: TR = 2000 ms; TE = 30 ms; slice thickness = 4 mm;gap = 0.5 mm; flip angle = 80◦; matrix size = 80 × 80; reconstruction matrix = 128 × 128, 1.875 × 1.875 mm; NEX = 1; number of slices = 26; and total acquisition time = 6 min. Total acquisition protocol consisted of 180 EPI dynamic volumes. We asked subjects to fixate on a target during resting-state acquisition. T1-weighted scans (3DFSPGR sequence) used the following parameters: 160 axial slices for whole brain coverage, TR = 10.91 ms; TE = in phase 4.2 ms; thickness = 1.2 mm; flip angle = 15◦; matrix size = 256 × 192; FOV = 24.0 × 18.0 cm; and NEX = 1.

Data preprocessing

Data were preprocessed using AFNI (version 2011_12_21_1014) and FSL software (version 5.0). We followed this stepwise procedure[21]: discard the first four volumes of EPI; skull stripping; head motion correction; despiking; rescale to a grand mean of 10000; band-pass filtering using classical resting-state band (0.01and 0.1 Hz); detrending; spatial smoothing (FWHM = 8 mm); linear registration to the subject's structural scan; structural image non-linear registration to the Montreal Neurological Institute (MNI152) template; non-linear registration of functional scans; and regression out of nuisance covariates (CSF, white matter, global signal and six linear motion parameters).

Head Movement

To minimize bias from head motion[22], we excluded subjects whose data did not pass quality-control thresholds (see Supplemental Material). We then applied the Power et al. (2012)[23] scrubbing method by discarding scans in which the frame-wise displacement (FD) exceeded 0.5 mm (see equation 9, from Yan et al (2013)[24, 25]). We

also entered the number of discarded volumes per subject from this scrubbing procedure as a covariate in all adjusted models. Finally, we performed a sensitivity analysis excluding subjects with more than 30 scrubbed volumes. Please note, no subject had mean FD > 0.3mm after scrubbing.

Statistical Analysis

Analyses were conducted using SPSS version 22 for Windows and R version 2.15.3; figure templates were created in MRICRON Software. All analyses were two-tailed, with significance threshold set at 5%.

Reward Network

First, we selected spheres centered at coordinates (in Montreal Neurological Institute space) reported by Bartra et al.'s (2013)[26] meta-analysis of the valuation system (Table S1). We defined the 11 ROIs in Satterthwaite et al. (2015).[10] The sphere radius for the ROIs was set at 5mm. We used Spearman´s coefficients to evaluate the correlation of the preprocessed (and scrubbed) BOLD signal between each pair of ROIs. This procedure created a matrix for each subject with 55 ROI-to-ROI correlations. We applied Fisher´s z transformation (0.5*log(1+r)/(1-r)) to the correlation coefficients. Then, we divided the sample by the site of data acquisition, creating two sub-samples, a discovery and replication sample: site 1 (n=328, Discovery), and site 2 (n=309, Replication). At site 1, we performed 55 one-sample t-tests to identify correlations connecting each pair of the 11 ROIs that were statistically different from 0. We used Bonferroni correction (p-value<.05/55 = .00091) to account for multiple comparisons. Then, we confirmed the Bonferroni-corrected significant findings in the independent Replication sample (site 2) with an uncorrected threshold (p<.05). Following convention[27], we termed these ROI-ROI correlations the edges of the reward network (43 edges, Table S2). We then computed reward-network connectivity measures among these 11 nodes by summing the absolute values of edges that survived the discovery and replication procedure connected to every given node. This measure is classically referred to as the weighted node degree centrality or the node strength[9], and reflects the importance of a specific node within the network.

Reward Network and Depressive Disorder

After probing reward network edges and nodes in discovery and replication samples, we proceeded to investigate the role of the reward network in adolescent depressive disorder using the entire sample, while also controlling for data-acquisition site. We used logistic regression to test the effect of left and right ventral striatum node

strength (independent variable) as predictor for depressive disorder (dependent variable) using the DSM-based clinician rating. We controlled for the following nuisance independent variables: number of scrubbed volumes, site, sex, age, and any anxiety disorder, attention-deficit/hyperactivity disorder (ADHD), and depressive disorder at baseline. The analysis survived Bonferroni correction for laterality (p-value 0.05/2). Then, we restricted our analysis to new-onset depressive disorder by running the same model whilst excluding depressive disorder (n=22) at baseline.

We further investigated the specificity of ventral striatum node strength as a predictor of depressive disorder:

a) First, we broadened our hypothesis-driven focus on ventral striatum and tested the node strength of all 11 nodes of the reward network as predictors for depressive disorder. We used logistic regression models to test node strength (independent variable) of reward nodes as predictors for depressive disorder (dependent variable), while controlling for number of scrubbed volumes, site, sex, age, and any anxiety disorder, attention-deficit/hyperactivity disorder (ADHD), and depressive disorder at baseline as nuisance independent variables.

b) We assessed diagnostic specificity by testing whether ventral striatum node strength predicts anxiety disorders, ADHD, or ASU (by parent- and self-report). We performed logistic regressions as in Aim 1, using as outcomes the clinician rating variables from DAWBA for anxiety disorders and ADHD, and ASU (by parent- and self-report). These models also included number of scrubbed volumes, site, sex, age, and psychopathology at baseline (any anxiety disorder, ADHD, and depressive disorder according to DAWBA clinician rating) as nuisance controlling variables.

4.2.4 Results

The prevalence of depressive disorder was 4.2% (n=27; MDD=25, Other Depression=2) at baseline and 8.8% (n=56; MDD=47, Other Depression=9) at follow-up. Predictors for depressive disorder at follow-up were female sex, older age, depressive disorder or ADHD at baseline. Additionally, baseline anhedonia significantly predicted depressive disorder at follow-up (OR 3.00, 95%CI 1.34-6.60, p=.01). At follow-up, older age, ADHD, any anxiety disorder, and ASU, by parent- and self-report, were all associated to depressive disorder (Table 1).

Table 4.2.1 Demographic and Clinical Characteristics of the HRC Study Participants

	No Depressive Disorder at follow-up (n=529)	Depressive Disorder at follow-up (n=56)	p-value
Baseline	n (%)	n (%)	
Sociodemographic			
Sex, F/M	241/288 (45.6/54.4)	37/19 (66.1/33.9)	.003
Site, Porto Alegre City/São Paulo City	274/255 (51.8/48.2)	35/21 (62.5/37.5)	.127
Age at MRI Scan, mean (SD), y	10.6 (1.9)	11.6 (1.8)	<.001
Maternal education – completed high school, Y/N [a]	226/298 (43.1/56.9)	26/30 (46.4/53.6)	.636
Socioeconomic score, mean (SD)	20.1 (4.4)	20.5 (5.6)	.879
Movement Parameters			
FD, mean (SD), mm (Pre-Scrubbing)	0.16 (0.23)	0.21 (0.34)	.623
Number of scrubbed volumes, mean (SD)	17.0 (27.1)	22.5 (32.7)	.354
FD, mean (SD), mm (Pos-Scrubbing)	0.08 (0.04)	0.09 (0.04)	.647
Clinical Features at baseline			
Any anxiety disorder, Y/N	75/454 (14.2/85.8)	13/43 (23.2/76.8)	.072
ADHD, Y/N	55/474 (10.4/89.6)	11/45 (19.6/80.4)	.038
Depressive Disorder, Y/N	12/519 (2.3/97.7)	12/44 (21.4/78.6)	<.001
3-year follow-up	n (%)	n (%)	
Time between MRI and FUP, mean (SD), y	2.6 (0.4)	2.5 (0.4)	.168
Any Anxiety Disorder, Y/N	59/470 (11.2/88.8)	26/30 (46.4/53.6)	<.001
ADHD, Y/N	21/508 (4.0/96.0)	6/50 (10.7/89.3)	.022
Any Substance Use by parent-report, Y/N [a]	79/437 (15.3/84.7)	22/32 (40.7/59.3)	<.001
Any Substance Use by self-report, Y/N [b]	192/279 (59.2/40.8)	32/17 (65.3/34.7)	<.001

[a] missing for 5 subjects;[b] missing for 65 subjects. Chi-square for categorical Variables; T-Test for scale variables and Mann-Whitney Test for scale variables not normally distributed. Abbreviations: F/M, female/male; SD, standard deviation; FD, frame displacement; ADHD, attention-deficit/hyperactivity disorder; FUP, follow-up; Y/N, yes/no.

Reward Network

We first identified (using Bonferroni correction) and replicated 43 significant correlations, i.e. edges, connecting the 11 nodes of the reward network (Table S2). We then created reward-network iFC measures of node strength among these 11 nodes by summing edges connected to every given node.

Table 4.2.2 Depressive Disorder by clinical rating at 3-year follow-up and Left Ventral Striatum Node Strength

	Outcome: Depressive Disorder at Follow-up (Exposed, n=529; Event, n=56)			
Variables in the model	OR	95% CI		p-value
Left ventral striatum node strength	1.54	1.09	to 2.18	.015
Depressive disorder at baseline	14.07	5.16	to 38.50	<.001
ADHD at baseline	2.06	.91	to 4.64	.081
Any anxiety at baseline	1.21	.54	to 2.73	.639
Age at MRI	1.45	1.22	to 1.74	<.001
Sex (female)	2.38	1.27	to 4.45	.007
Site	1.16	.59	to 2.29	.6667
Number of scrubbed volumes [a]	1.01	.99	to 1.02	.342

[a] Movement parameter. Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; ADHD, attention-deficit/hyperactivity disorder; MRI, magnetic resonance imaging.

Reward Network and Depressive Disorder

Our main aim was to test whether ventral striatum node strength predicted risk for depressive disorder over 3 years. We confirmed this hypothesis for the left ventral striatum node, which is connected to ACC, PFC, Thalamus and ventral tegmental area, among other reward network regions (Fig 1). This result was significant both in bivariate analysis and in an analysis that controlled for potential baseline confounders (OR 1.54, 95%CI=1.09-2.18, p=.03, corrected for bilateral striatum) (Table 2), including anxiety, ADHD and depressive disorder. No association existed for the right ventral striatum node (OR 1.23, 95%CI 0.83-1.82, p=.311) (Table 3). Elevated node strength of the left ventral striatum node predicted a 50% increase in the odds of a depressive disorder 3 years later. We also found similar results when excluding depressive disorder at baseline (OR 1.52, 95%CI 1.05-2.20, p=.027). Ventral striatum node strength was not significantly associated to depressive disorder or to anhedonia at baseline (data available upon request). Also, the main results did not change when we conducted sensitivity analyses that excluded subjects who had more than 30 volumes eliminated by the scrubbing procedure (Table S3).

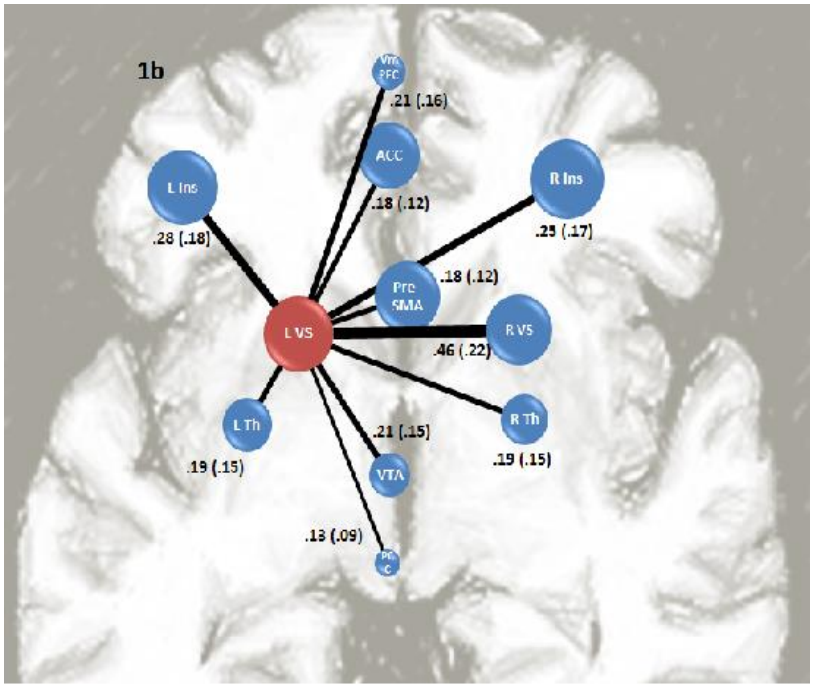

Figure 4.2.1 Schematic Representation of Edges Connecting Reward Nodes to the Left Ventral Striatum 1a. 3D representation of left ventral striatum node and other nodes of the reward network in MNI space. 1b. Mean correlation coefficients (standard deviation) of resting-state time-series between reward network node and the left ventral striatum node. Width of lines is proportional to the correlation coefficient (edges), and size of the circles is proportional to node strength within the reward network. 1c. Axial view of actual size and position of reward network node in MNI Space. For abbreviations, see Table 4.2.S1

Specificity Analyses

We assessed the specificity of left ventral striatum node as a predictor of depressive disorder in two ways: (a) comparing it with other reward nodes and (b) as a predictor of other common adolescent psychopathology.

a) We tested all other node strength of the reward network as predictors of depressive disorder. Only the left ventral striatum node strength significantly predicted depressive disorder (Table 3).

b) Second, we tested whether left ventral striatum node strength predicted adolescent psychopathology other than depressive disorder. Left ventral striatum was not associated with any anxiety disorder, ADHD, or ASU (by parent- or self-report) at 3-year follow-up (Table S4). Therefore, compared to other common adolescent disorders, the association between left ventral striatum iFC and psychopathology 3 years later was indeed specific to depressive disorder.

Table 4.2.3 Depressive Disorder by clinical rating at 3-year follow-up and Node Strength of all Reward Network Nodes.

	Outcome: Depressive Disorder at Follow-up (Exposed, n=529; Event, n=56)		
Nodes	OR	95% CI	p-value

Striatal Node Strength			
Left Ventral Striatum	1.54	1.09 to 2.18	.015*
Right Ventral Striatum	1.23	.83 to 1.82	.311
Other Regions from the Reward Network			
Ventromedial Prefrontal Cortex	.81	.41 to 1.59	.534
Left Anterior Insula	1.21	.76 to 1.66	.569
Right Anterior Insula	1.33	.90 to 1.98	.162
Posterior Cingulate	1.16	.48 to 2.85	.742
Brainstem (Ventral Tegmental Area)	.93	.56 to 1.55	.775
Anterior Cingulate	1.02	.65 to 1.58	.948
Pre-Supplementary motor area	1.18	.76 to 1.84	.452
Left Thalamus	1.03	.64 to 1.65	.904
Right Thalamus	1.16	.72 to 1.87	.542

* Corrected for striatum laterality (p/2) p=.03. All models controlled for sex, age, site, number of scrubbed volumes, and the following psychiatric disorder at baseline: any anxiety disorder, any attention-deficit/hyperactivity disorder, and depressive disorder. Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

4.2.5 Discussion

Using a community-based sample, we found that ventral striatal iFC predicted new onset depressive disorder three years later. We also found evidence of specificity, i.e., connectivity of the striatum, but not other regions, predicted depressive disorder; and striatal connectivity predicted depressive disorder, but not other psychopathology. The results of our longitudinal design provide novel evidence for the involvement of the reward network to the pathogenesis of depression.

Several clinical and basic-science considerations implicate reward processing in depression. From a clinical perspective, there is the long-standing observation that a subset of depressive behaviors, such as reduced energy and motivation, are related to changes in reinforcement schedules.[28, 29] These notions underpin therapeutic approaches, in particular behavioral activation, a key component of cognitive and behavioral therapy in depression.[30] These observations converge with basic-science findings about reward processing. Several experiments demonstrate the key role that dopaminergic signaling in ventral striatal areas plays in reward valuation and effort expended towards reward.[31, 32] In recent years, fMRI has enabled scientists to probe activity in deep brain areas such as the ventral striatum and therefore provide crucial links between long-standing clinical notions and basic science. Indeed, there is mounting evidence from reward task-based fMRI that reduced activity in the striatum is important in depression etiology.[3, 5, 33] However, there is a need to understand the

distributed brain patterns of perturbations in depression[6], and network measures using iFC are well suited to this approach. Unlike task-based fMRI, iFC does not rely on a behavioral paradigm and is therefore less confounded by issues such as ability or motivation to engage with a task.[34] This is particularly important when studying developmental effects where standardizing a task across different age groups can present a daunting challenge. Therefore, since both reward processing and depression prevalence vary with development[2, 35, 36], iFC appears well suited to their study.

Probing the connectivity of a reward network allowed us to show that increased left ventral striatum node strength predicts depressive disorder at follow-up. Our first step was to show resting-state coupling between brain regions typically activated during reward-related behaviors.[3, 26, 37] Then, we computed node strength – an important network measure that captures the centrality of a given node within a network.[9] The left striatum was the only node whose strength predicted depressive disorder. This suggests that left ventral striatum is integrating information from various areas of the reward network, including those previously implicated in adolescent depression, such as the ACC.[13, 14] Having probed rigorously ventral striatal iFC within the reward network, we then used it in the prediction of depressive disorder 3 years later. We demonstrate that left ventral striatum node strength predicts new onset depressive disorder, that is, even after excluding depressive disorder cases at baseline.

This finding indicates that perturbed connectivity in the reward network is not merely a consequence of experiencing depression, but predates the expression of the disorder. Thus, striatal iFC is a marker of depressive disorder risk and supports its role in the pathogenesis of depression, although our observational study cannot offer conclusive evidence about its causal role (i.e. striatal iFC could still be an early marker, but not be itself implicated in the illness). It should be noted that we did not find a significant association between striatal node strength and depressive disorder at baseline. However, the low prevalence of depressive disorder at baseline, which is expected given the young age of participants at that point, may have diminished statistical power to demonstrate this association.

Our study finds that increased, rather than decreased, iFC predicts depressive disorder. One possible explanation for this finding is that increased iFC is an attempt at compensating for the blunted striatal response to rewards that has been described in depression.[6] Coupling resting state connectivity studies with functional imaging

probing the ventral striatum could help test this hypothesis. Alternatively, hyperconnectivity within the reward network could reflect a primary pathogenic process in its own right. Resembling hyperconnectivity within other networks found in depression studies, such as the Default Mode Network[7], increased iFC may itself impede adequate reward processing during reward-related tasks, leading to blunted ventral striatum signals; a hypothesis which could also be tested in longitudinal studies that employ serial resting-state and task-based fMRI studies. A previous study found decreased iFC within the reward network[10], yet this discrepancy could be explained by the fact that these were adult subjects who already had depression and, unlike our adolescents, were on medication.

Lastly, since psychiatric disorders in youth are frequently comorbid[38] and ventral striatal dysfunction is implicated in other disorders[39, 40], we examined whether left ventral striatum node strength could predict diagnoses other than depressive disorder. Supporting the specificity of our main result, the node strength of the left ventral striatum did not significantly predict anxiety disorders and ADHD. We also investigated the association of the left ventral striatum node with another reward-related phenotype, any substance use. One prior longitudinal study showed that stronger cortico-striatal iFC (right dorsolateral PFC, dorsomedial PFC, and pre-SMA) predicted earlier onset of alcohol and substance use.[40] We did not find this association. However, our sample was younger than the expected age ASU onset. Further evidence that this is a limitation for detecting ASU findings is that age was positively associated with ASU (Table S4). Future work dissecting the various anatomical and functional components of reward processing may identify differential predictions between MDD and other disorders, such as substance-related and addictive disorders.

Our study has a number of strengths. Our discovery and replication analysis addresses recent concerns regarding the lack of replicability in neuroimaging studies.[25] Furthermore, adolescent MDD studies typically rely on cross-sectional designs and relatively small clinically-referred samples, whereas ours was a longitudinal study in a large community-based sample of unmedicated adolescents. However, our study also has limitations. We investigated a specific brain network, despite evidence of several networks being implicated in both MDD and typical development.[6, 7] The main advantage of this approach, which narrows brain regions based on prior data, is to avoid spurious associations found in whole-brain investigations. In addition, there was attrition at follow up, which can introduce bias. However, our loss at follow up was in

the better range (10% of the imaging sample). Also, adolescence and young adulthood is the age of maximum incidence of depression. This may have led to an underestimation of the strength of our effects since most subjects in our study were in their early adolescence. Moreover, we did not collect child reports of depression at baseline because of the low reliability of youth report in early childhood.[18, 20] Finally, iFC data are sensitive to head motion.[22, 23] We addressed this issue using distinct techniques and rigorous thresholding. Importantly, our main results persisted after imposing restrictive head movement parameters, therefore increasing confidence in our findings.

In sum, we investigated the iFC of the ventral striatum within the reward network in a community sample of adolescents. Increased ventral striatum node strength increased the odds of depressive disorder by approximately 50% after 3 years. This underscores the importance of the brain's reward network in the pathogenesis of depression and calls for further studies to make clinical use of these findings.

4.2.6 References

1. Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJ, et al. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. PLoS Med. 2013;10(11):e1001547.

2. Beesdo K, Hofler M, Leibenluft E, Lieb R, Bauer M, Pfennig A. Mood episodes and mood disorders: patterns of incidence and conversion in the first three decades of life. Bipolar disorders. 2009;11(6):637-49.

3. Forbes EE, Dahl RE. Research Review: altered reward function in adolescent depression: what, when and how? Journal of child psychology and psychiatry, and allied disciplines. 2012;53(1):3-15.

4. Stringaris A, Vidal-Ribas Belil P, Artiges E, Lemaitre H, Gollier-Briant F, Wolke S, et al. The Brain's Response to Reward Anticipation and Depression in Adolescence: Dimensionality, Specificity, and Longitudinal Predictions in a Community-Based Sample. The American journal of psychiatry. 2015;172(12):1215-23.

5. Kerestes R, Davey CG, Stephanou K, Whittle S, Harrison BJ. Functional brain imaging studies of youth depression: a systematic review. NeuroImage Clinical. 2014;4:209-31.

6. Mulders PC, van Eijndhoven PF, Schene AH, Beckmann CF, Tendolkar I. Resting-state functional connectivity in major depressive disorder: A review. Neuroscience and biobehavioral reviews. 2015;56:330-44.

7. Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA. Large-Scale Network Dysfunction in Major Depressive Disorder: A Meta-analysis of Resting-State Functional Connectivity. JAMA Psychiatry. 2015;72(6):603-11.

8. Drysdale AT, Grosenick L, Downar J, Dunlop K, Mansouri F, Meng Y, et al. Resting-state connectivity biomarkers define neurophysiological subtypes of depression. Nat Med. 2017;23(1):28-38.

9. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. Neuroimage. 2010;52(3):1059-69.

10. Satterthwaite TD, Kable JW, Vandekar L, Katchmar N, Bassett DS, Baldassano CF, et al. Common and Dissociable Dysfunction of the Reward System in Bipolar and Unipolar Depression. Neuropsychopharmacology. 2015;40(9):2258-68.

11. Sharma A, Wolf DH, Ciric R, Kable JW, Moore TM, Vandekar SN, et al. Common Dimensional Reward Deficits Across Mood and Psychotic Disorders: A Connectome-Wide Association Study. The American journal of psychiatry. 2017:appiajp201616070774.

12. Gabbay V, Ely BA, Li Q, Bangaru SD, Panzer AM, Alonso CM, et al. Striatum-based circuitry of adolescent depression and anhedonia. Journal of the American Academy of Child and Adolescent Psychiatry. 2013;52(6):628-41.e13.

13. Davey CG, Whittle S, Harrison BJ, Simmons JG, Byrne ML, Schwartz OS, et al. Functional brain-imaging correlates of negative affectivity and the onset of first-episode depression. Psychological medicine. 2015;45(5):1001-9.

14. Connolly CG, Wu J, Ho TC, Hoeft F, Wolkowitz O, Eisendrath S, et al. Resting-state functional connectivity of subgenual anterior cingulate cortex in depressed adolescents. Biological psychiatry. 2013;74(12):898-907.

15. Cullen KR, Westlund MK, Klimes-Dougan B, Mueller BA, Houri A, Eberly LE, et al. Abnormal amygdala resting-state functional connectivity in adolescent depression. JAMA Psychiatry. 2014;71(10):1138-47.

16. Salum GA, Gadelha A, Pan PM, Moriyama TS, Graeff-Martins AS, Tamanaha AC, et al. High risk cohort study for psychiatric disorders in childhood: rationale, design, methods and preliminary results. International journal of methods in psychiatric research. 2015;24(1):58-73.

17. Weissman MM, Wickramaratne P, Adams P, Wolk S, Verdeli H, Olfson M. Brief screening for family psychiatric history: the family history screen. Archives of general psychiatry. 2000;57(7):675-82.

18. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. Journal of child psychology and psychiatry, and allied disciplines. 2000;41(5):645-55.

19. Fleitlich-Bilyk B, Goodman R. Prevalence of child and adolescent psychiatric disorders in southeast Brazil. Journal of the American Academy of Child and Adolescent Psychiatry. 2004;43(6):727-34.

20. Schwab-Stone M, Fallon T, Briggs M, Crowther B. Reliability of diagnostic reporting for children aged 6-11 years: a test-retest study of the Diagnostic Interview Schedule for Children-Revised. The American journal of psychiatry. 1994;151(7):1048-54.

21. Mennes M, Biswal BB, Castellanos FX, Milham MP. Making data sharing work: the FCP/INDI experience. Neuroimage. 2013;82:683-91.

22. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. Neuroimage. 2014;84:320-41.

23. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage. 2012;59(3):2142-54.

24. Yan CG, Cheung B, Kelly C, Colcombe S, Craddock RC, Di Martino A, et al. A comprehensive assessment of regional variation in the impact of head micromovements on functional connectomics. Neuroimage. 2013;76:183-201.

25. Sato JR, Biazoli CE, Salum GA, Gadelha A, Crossley N, Vieira G, et al. Connectome hubs at resting state in children and adolescents: Reproducibility and psychopathological correlation. Developmental Cognitive Neuroscience. 2016;20:2-11.

26. Bartra O, McGuire JT, Kable JW. The valuation system: a coordinate-based meta-analysis of BOLD fMRI experiments examining neural correlates of subjective value. Neuroimage. 2013;76:412-27.

27. Sato JR, Salum GA, Gadelha A, Picon FA, Pan PM, Vieira G, et al. Age effects on the default mode and control networks in typically developing children. Journal of psychiatric research. 2014;58:89-95.

28. Lewinsohn PM, Sullivan JM, Grosscup SJ. Changing Reinforcing Events - an Approach to the Treatment of Depression. Psychother-Theor Res. 1980;17(3):322-34.

29. Jacobson NS, Martell CR, Dimidjian S. Behavioral activation treatment for depression: Returning to contextual roots. Clin Psychol-Sci Pr. 2001;8(3):255-70.

30. Dimidjian S, Hollon SD, Dobson KS, Schmaling KB, Kohlenberg RJ, Addis ME, et al. Randomized trial of behavioral activation, cognitive therapy, and antidepressant medication in the acute treatment of adults with major depression. J Consult Clin Psychol. 2006;74(4):658-70.

31. Ferenczi EA, Zalocusky KA, Liston C, Grosenick L, Warden MR, Amatya D, et al. Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. Science (New York, NY. 2016;351(6268):aac9698.

32. Treadway MT, Buckholtz JW, Cowan RL, Woodward ND, Li R, Ansari MS, et al. Dopaminergic Mechanisms of Individual Differences in Human Effort-Based Decision-Making. Journal of Neuroscience. 2012;32(18):6170-6.

33. Sonuga-Barke EJ, Cortese S, Fairchild G, Stringaris A. Annual Research Review: Transdiagnostic neuroscience of child and adolescent mental disorders--differentiating decision making in attention-deficit/hyperactivity disorder, conduct disorder, depression, and anxiety. Journal of child psychology and psychiatry, and allied disciplines. 2016;57(3):321-49.

34. Helfinstein SM, Kirwan ML, Benson BE, Hardin MG, Pine DS, Ernst M, et al. Validation of a child-friendly version of the monetary incentive delay task. Soc Cogn Affect Neurosci. 2013;8(6):720-6.

35. van Duijvenvoorde AC, Achterberg M, Braams BR, Peters S, Crone EA. Testing a dual-systems model of adolescent brain development using resting-state connectivity analyses. Neuroimage. 2016;124(Pt A):409-20.

36. Braams BR, van Duijvenvoorde AC, Peper JS, Crone EA. Longitudinal changes in adolescent risk-taking: a comprehensive study of neural responses to rewards, pubertal development, and risk-taking behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2015;35(18):7226-38.

37. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. Neuropsychopharmacology. 2010;35(1):4-26.

38. Kessler RC, Avenevoli S, McLaughlin KA, Green JG, Lakoma MD, Petukhova M, et al. Lifetime co-morbidity of DSM-IV disorders in the US National Comorbidity Survey Replication Adolescent Supplement (NCS-A). Psychological medicine. 2012;42(9):1997-2010.

39. Ma I, van Holstein M, Mies GW, Mennes M, Buitelaar J, Cools R, et al. Ventral striatal hyperconnectivity during rewarded interference control in adolescents with ADHD. Cortex; a journal devoted to the study of the nervous system and behavior. 2016;82:225-36.

40. Pannekoek JN, van der Werff SJ, van Tol MJ, Veltman DJ, Aleman A, Zitman FG, et al. Investigating distinct and common abnormalities of resting-state functional connectivity in depression, anxiety, and their comorbid states. Eur Neuropsychopharmacol. 2015;25(11):1933-42.

4.2.7 Supplemental Material

Neuroimaging Procedures from High Risk Cohort

The HRC project aimed to evaluate 750 children using Magnetic Resonance Imaging (MRI). This number was set in accordance to the project budget. Therefore, the procedure involved inviting the first set of subjects who had successfully completed the household interview, based on the dates in which they were enrolled in the project. We screened the first 1159 subjects who completed the parent- and self-report at baseline; 136 (11.5%) refused to participate in a phone interview for MRI eligibility, and 59 (5.0%) had reported using braces (n=19) or screened positive for medical restriction (n=40). Application of these rules led us to evaluate 964 eligible subjects. From these 964, 876 (90.9%) met criteria for scanning and were scheduled, 38 (3.9%) could not be contacted to schedule the scans in the allotted time, and 50 (5.2%) refused to attend to the MRI session. Finally, we acquired T1 and Resting-state fMRI data on 741 (76.9%) participants. No statistically-significant differences emerged for age (p=.634), sex (p=.391), site (p=.365), and socioeconomic status (p=.686) among eligible subjects who did and did not provide MRI data. Mother´s level of education was higher in subjects who provided MRI data (p=.047).

Before scanning, we trained participants to minimize head movement by desensitizing them to enclosed spaces and scanner noise in a simulated scanning

environment. From 741 subjects attending the MRI sessions, data were excluded for 86 subjects. These subjects included 9 (1.2%) with missing clinical data, 38 (5.1) who aborted the scan session, and 39 (5.3%) whose data contained artifacts or failed to pass quality-control procedures for other reasons (5.3%). Table S1a compares characteristics between the 86 (11.6%) excluded subjects and the remaining 655 (88.4%). Included subjects tended to be older (p=.063) and scanned at the Porto Alegre site. Table S1b compares baseline characteristics in subjects who did (n=585) or did not (n=52) complete the follow-up assessment. A higher proportion of subjects from the Porto Alegre site completed the follow-up (chi-square= 5.07; p=.024), but there were no other differences between these two groups.

Table 4.2.S1 Demographic and Clinical Characteristics of the HRC Study Participant: Exclusions and Losses at Follow-up

Table S1a. Demographic and clinical Characteristics of the HRC Study Participant: Exclusions at MRI

	Excluded: Failed to complete T1 and/or resting-state MRI (n=86) [a]	Successfully Completed T1 and resting-state MRI (n=655)	p-value
	n (%)	n (%)	
Sociodemographic at baseline			
Sex, F/M	31/49 (38.8/61.3)	311/344 (47.5/52.5)	.139
Site, Porto Alegre City/São Paulo City	25/55 (31.3/68.8)	342/313 (52.2/47.8)	<.001
Age at MRI Scan, mean (SD), y	10.2 (1.8)	10.7 (1.9)	.063
Maternal education – completed high school, Y/N [b]	29/49 (62.8/37.2)	283/363 (43.8/56.2)	.264
Socioeconomic score, mean (SD)	19.6 (5.6)	20.1 (4.5)	.173
Clinical Features at baseline			
Any anxiety disorder, Y/N	9/71 (11.3/88.8)	99/556 (15.1/84.9)	.357
ADHD, Y/N	9/71 (11.3/88.8)	80/575 (12.2/87.8)	.803
Depressive Disorder, Y/N	2/78 (2.5/97.5)	28/627 (4.3/95.7)	.449

Table S1b. Demographic and clinical Characteristics of the HRC Study Participant: Exclusions Due to Loss at Follow-up

	Excluded: Loss at Follow-up (n=52)	Successfully Completed Household Follow-up (n=585)	p-value
	n (%)	n (%)	
Sociodemographic at baseline			
Sex, F/M	24/28 (46.2/53.8)	278/307 (47.5/52.5)	.850
Site, Porto Alegre City/São Paulo City	19/33 (36.5/63.5)	309/276 (52.8/47.2)	.024
Age at MRI Scan, mean (SD), y	10.5 (2.1)	10.7 (1.9)	.425
Maternal education – completed high school, Y/N [c]	23/27 (46.0/54.0)	252/328 (46.4/56.6)	.727
Socioeconomic score, mean (SD)	19.8 (4.3)	20.2 (4. 6)	.710
Clinical Features at baseline			
Any anxiety disorder, Y/N	3/49 (5.8/94.2)	88/497 (15.0/85.0)	.067
ADHD, Y/N	6/46 (11.5/88.5)	66/519 (11.3/88.7)	.955
Depressive Disorder, Y/N	3/49 (5.8/94.2)	66/561 (4.1/95.9)	.568

Movement Parameters			
FD, mean (SD), mm (Pre-Scrubbing)	0.11 (0.10)	0.16 (0.24)	.075
Number of scrubbed volumes, mean (SD)	10.17 (17.3)	17.5 (27.7)	.111
FD, mean (SD), mm (Pos-Scrubbing)	0.08 (0.04)	0.08 (0.04)	.893

[a] Numbers vary due to missing data; [b] missing for 17 subjects; [c] missing for 7 subjects; chi-square for categorical variables; Mann-Whitney Test for scale variables not normally distributed. Abbreviations: F/M, female/male; SD, standard deviation; FD, frame displacement; ADHD, attention-deficit/hyperactivity disorder; FUP, follow-up; Y/N, yes/no.

Table 4.2.S2 Regions of Interest of the Reward Network – Montreal Neurological Institute (MNI) coordinates

ROI	X	Y	Z
Left Ventral Striatum (L VS)	-12	12	-6
Right Ventral Striatum (R VS)	12	10	-6
Ventromedial Prefrontal Cortex (VmPFC)	2	46	-8
Left Anterior Insula (L Ins)	-30	22	-6
Right Anterior Insula (R Ins)	32	20	-6
Posterior Cingulate (PCC)	-4	-30	36
Brainstem - Ventral Tegmental Area (VTA)	-2	-22	-12
Anterior Cingulate (ACC)	-2	28	28
Pre-Supplementary motor area (Pre-SMA)	-2	16	46
Left Thalamus (L Th)	-6	-8	6
Right Thalamus (R Th)	6	-8	6

Table 4.2.S3 Discovery and Replication of Correlations between Regions of Interest of the Reward Network

Edge (node-node)	Site 1 Discovery Bonferroni Corrected (p<.00091)		Site 2 Replication Uncorrected p (p<.05)	
	n = 328		n = 309	
	t	*p*	t	*p*
ACC-VTA	11.22	<.00001	13.52	<.00001
ACC-L Ins	28.92	<.00001	31.13	<.00001
ACC-L VS	15.75	<.00001	17.50	<.00001
ACCL Th	11.67	<.00001	13.00	<.00001
ACC-PCC	14.23	<.00001	13.79	<.00001
ACC-PreSMA	33.25	<.00001	30.63	<.00001
ACC-R Ins	28.11	<.00001	27.45	<.00001
ACC-R VS	11.31	<.00001	16.45	<.00001
ACC-R Th	8.98	<.00001	12.52	<.00001
ACC-VMPFC	3.91	.00011	-0.57	.57176
VTA-L Ins	16.99	<.00001	19.81	<.00001
VTA-L VS	14.90	<.00001	19.64	<.00001
VTA-L Th	1.60	.11086	12.31	<.00001
VTA-PCC	7.63	<.00001	6.66	<.00001
VTA-PreSMA	14.46	<.00001	14.64	<.00001
VTA-R Ins	17.50	<.00001	19.83	<.00001
VTA-R VS	12.85	<.00001	20.06	<.00001
VTA-R Th	-.01	.99193	13.03	<.00001
VTA-VMPFC	5.69	<.00001	-0.84	.40176
L Ins-L VS	22.56	<.00001	28.61	<.00001
L Ins-L Th	4.53	<.00001	13.85	<.00001
L Ins-PCC	4.03	.00007	-0.34	.73152
L Ins-PreSMA	34.04	<.00001	33.10	<.00001
L Ins-R Ins	43.98	<.00001	55.56	<.00001
L Ins-R VS	17.41	<.00001	27.16	<.00001
L Ins-R Th	1.21	.22668	10.72	<.00001
L Ins-VMPFC	9.08	<.00001	6.26	<.00001
L VS-L Th	6.41	<.00001	18.98	<.00001
L VS-PCC	8.13	<.00001	4.22	.00003
L VS-PreSMA	13.93	<.00001	18.21	<.00001
L VS-R Ins	19.28	<.00001	25.50	<.00001
L VS-R VS	30.66	<.00001	47.85	<.00001
L VS-R Th	4.63	<.00001	18.37	<.00001
L VS-VMPFC	15.49	<.00001	12.16	<.00001
L Th-R Th	43.58	<.00001	50.74	<.00001
PCC-L Th	-.94	.34719	6.07	<.00001
PCC-PreSMA	1.30	.19347	-2.45	.01474
PCC-R Ins	1.16	.24575	-0.39	.69931
PCC-R VS	3.08	.00224	3.67	.00028
PCC-R Th	-3.38	.00081	2.96	.00329
PCC-VMPFC	15.61	<.00001	12.16	<.00001
PreSMA-L Th	11.77	<.00001	14.38	<.00001
PreSMA-R Ins	26.64	<.00001	28.48	<.00001
PreSMA-R VS	9.93	<.00001	15.18	<.00001
PreSMA-R Th	8.11	<.00001	11.66	<.00001
PreSMA-VMPFC	-8.55	<.00001	-12.23	<.00001
R Ins-L Th	6.06	<.00001	8.33	<.00001
R Ins-R VS	20.44	<.00001	29.40	<.00001
R Ins-R Th	7.36	<.00001	12.40	<.00001
R Ins-VMPFC	6.62	<.00001	6.29	<.00001
R VS-L Th	6.63	<.00001	17.46	<.00001
R VS-R Th	7.39	<.00001	19.45	<.00001
R VS-VMPFC	12.22	<.00001	13.10	<.00001
VMPFC-L Th	-4.93	<.00001	0.87	.38514
VMPFC-R Th	-6.05	<.00001	-0.18	.85705

Note: For abbreviations see Table S1

Table 4.2.S4 Logistic Regression model. Depressive Disorder by clinical rating at 3-year follow-up and Node Strength of the Left Ventral Striatum Within the Reward Network Excluding Subjects with more than 30 Excluded Volumes after Scrubbing Procedure.

Variables in the model	Outcome: MDD at Follow-up (Exposed. n=426; Event. n=40)		
	OR	95% CI	p-value
Left ventral striatum iFC	1.94	1.20 to 3.14	.007
Depressive disorder at baseline	13.83	4.33 to 44.18	<.001
ADHD at baseline	1.91	.71 to 5.19	.202
Any anxiety at baseline	1.59	.63 to 4.00	.329
Age at MRI	1.53	1.24 to 1.90	<.001
Sex (female)	1.92	.92 to 4.02	.082
Site	.95	.42 to 2.16	.903
Number of Scrubbed Volumes[a]	1.00	.95 to 1.05	.901

[a] Movement Parameter. . Abbreviations: MDD. major depressive disorder; OR. odds ratio; 95% CI. 95% confidence interval; ADHD. attention-deficit/hyperactivity disorder; MRI. magnetic resonance imaging.

Table 4.2.S5 Left Ventral Striatum Node Strength as a Predictor for Common Adolescent Psychiatric Outcomes

Table S4a. Logistic Regression model: any anxiety by clinician rating at 3-year follow-up predicted by Left Ventral Striatum Node Strength at Baseline

	Outcome: any anxiety at follow-up (Exposed. n=500; Event. n=85)		
Variables in the model	OR	95% CI	p-value
Left ventral striatum node strength	.77	.56 to 1.07	.119
Depressive disorder at baseline	1.86	.70 to 4.91	.212
ADHD at baseline	1.18	.57 to 2.41	.659
Any anxiety at baseline	2.48	1.38 to 4.43	.002
Age at MRI	.98	.86 to 1.11	.718
Sex (female)	1.60	.99 to 2.57	.053
Site	1.32	.78 to 2.22	.296
Number of Scrubbed Volumes[a]	1.00	.99 to 1.01	.814

Table S4b. Logistic Regression model: ADHD by clinician rating at 3-year follow-up predicted by Left Ventral Striatum Node Strength at Baseline

	Outcome: ADHD at follow-up (Exposed. n=558; Event. n=27)		
Variables in the model	OR	95% CI	p-value
Left ventral striatum node strength	1.51	.96 to 2.38	.078
Depressive disorder at baseline	.77	.09 to 6.54	.810
ADHD at baseline	7.51	3.23 to 17.47	.000
Any anxiety at baseline	.60	.18 to 2.01	.408
Age at MRI	.87	.69 to 1.11	.265
Sex (female)	.77	.33 to 1.79	.542
Site	.53	.21 to 1.30	.165
Number of Scrubbed Volumes[a]	.99	.98 to 1.01	.501

Table S4c. Logistic Regression model: Any Substance Use by parent report at 3-year follow-up predicted by Left Ventral Striatum Node Strength at Baseline

	Outcome: Parent-Report Any Substance Use[b] (Exposed. n=469; Event. n=101)		
Variables in the model	OR	95% CI	p-value
Left ventral striatum node strength	1.06	.78 to 1.43	.721
Depressive disorder at baseline	2.62	.97 to 7.09	.057
ADHD at baseline	1.52	.77 to 2.98	.229
Any anxiety at baseline	.97	.51 to 1.87	.936
Age at MRI	1.65	1.43 to 1.90	<.001
Sex (female)	1.15	.72 to 1.85	.552
Site	.48	.28 to.82	.007

Number of Scrubbed Volumes[a]	1.00	.99 to 1.01	.418

Table S4c. Logistic Regression model: Any Substance Use by parent report at 3-year follow-up predicted by Left Ventral Striatum Node Strength at Baseline

	Outcome: Self-Report Any Substance Use[c] (Exposed. n=296; Event. n=224)		
Variables in the model	OR	95% CI	p-value
Left ventral striatum node strength	.99	.76 to 1.30	.967
Depressive disorder at baseline	1.20	.39 to 3.68	.754
ADHD at baseline	.54	.27 to 1.05	.069
Any anxiety at baseline	1.15	.64 to 2.06	.640
Age at MRI	1.90	1.67 to 2.16	<.001
Sex (female)	1.03	.68 to 1.54	.903
Site	.59	.38 to .92	.020
Number of Scrubbed Volumes[a]	.99	.98 to 1.00	.111

[a] Movement Parameter; [b] 15 missing values for this variable; [c] 117 missing values for this variable. Abbreviations: OR. odds ratio; 95% CI. 95% confidence interval; ADHD. attention-deficit/hyperactivity disorder; MRI. magnetic resonance imaging.

4.3 Study 3: Psychotic Experiences and Common Mental Disorders in childhood and early adolescence: bidirectional and transdiagnostic associations in a longitudinal community-based study

This paper is in preparation for submission to Schizophrenia Bulletin.
Impact Factor - 7.58 – 2016 Journal Citation Reports.(69)
ISSN - 0586-7614- Qualis A1, MEDICINA II (2013-2016).

4.3.1 Authors and Abstract

Pedro Mario Pan[1], INPD-HRC Research Group, Rodrigo A. Bressan[1,2], M.D., Ph.D.

[1]Deparment of Psychiatry, Laboratório Interdisciplinar de Neurociências Clínicas (LiNC), Universidade Federal de São Paulo, São Paulo, Brazil.

[2]Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London.

Psychotic Experiences (PE) in early adolescence may increase the risk for later psychotic and non-psychotic disorders, and the presence of common psychiatric disorders may also increase the risk of PE later in life. Here, we aim to explore bidirectional associations of PE and youth common mental disorder in a 3-year follow-up community-based study. At baseline, we evaluated 2,244 6-12 years old subjects using dimensional and categorical measures of PE by self-report and clinician rating. We merged mental disorders into 4 DSM-based groups: any depressive disorder, any anxiety disorder, any Attention Deficit Hyperactivity Disorder (ADHD), and any Oppositional Defiant Disorder or Conduct Disorder (ODD/CD). Subjects were reassessed with the same instruments after 3 years. Logistic regression models tested the association of PE and each mental disorder group. Poisson regression determined whether PE predicted the number of comorbid disorders, a proxy for a nonspecific "psychiatric load/liability". We found bidirectional associations between PE and youth mental disorders. Baseline PE increased the risk of any depressive disorder at follow-up, whereas baseline ADHD was associated with PE at 3-year follow-up. Comorbidity

analyses showed significant relationships in both directions, with an increased risk of PE according to the number of comorbid psychiatric disorders in the opposite time point. We showed that subthreshold psychotic symptoms predict subsequent depressive disorder, and nonspecifically relate to psychiatric comorbidity. These findings are concordant with the notion that psychotic experiences are part of the same psychiatric vulnerability conferred to common mental disorders, such as depression and ADHD.

4.3.2 Introduction

Psychotic Experiences (PE) refers to subthreshold psychotic symptoms in the general population, such as delusion-like thoughts and perceptual aberrances, which can lead to distress and impairment.[1, 2] There are studies, for instance, connecting PE to increased risk of suicidality and self-harm[3, 4], violence perpetration and arrest[3, 5], mental health service use[6], and all-cause mortality.[7] Previous research has linked PE in early adolescence with schizophrenia and other psychotic disorders, showing the deleterious role of persistent PE.[8-11] However, few subjects have persistent PE, and among those, fewer make the transition to a psychotic disorder.[1, 9-12] Several studies, on the other hand, report on the association of PE with non-psychotic disorders, and subjects with psychiatric disorders have increased rates of PE when compared to controls.[8, 13-17] However, no previous research studied the bidirectional associations between mental disorders and PE in the transition from childhood to adolescence. The investigation of PE in this is age range is crucial given the evolving elements of neurodevelopment and psychopathology.[18-22]

Previous investigations linked PE with later non-psychotic disorders.[8, 10, 11, 14, 23] PE at age 11 increased the likelihood of meeting criteria for a lifetime psychiatric disorder at age 38.[8] Longitudinal studies found that adolescent PE was a predictor of later mood and anxiety disorders[10, 11, 24], but this finding was not confirmed in the pooled result of a meta-analysis including 5 large longitudinal samples.[10] Interestingly, few studies assessed disorders that typically emerge during development, such as Attention Deficit Hyperactivity Disorder (ADHD), Oppositional Defiant Disorder (ODD), and Conduct Disorder (CD). In this direction, a recent report from the Multimodal Treatment Study of Children with ADHD found similar levels of PE in ADHD *vs* control subjects.[25] In addition, McGrath et al (2016)[14] analyzed data from the World Mental Health survey, a large cross-national adult sample, and did not find significant

associations between PE and later ADHD, ODD, or CD. However, few cases reported PE before the onset of these disorders, which may have affected the statistical power to show significant associations. Moreover, the cross-sectional design limits interpretations of causality due to recall bias, particularly for childhood-onset disorders. Therefore, further investigations of the longitudinal relationship between PE and youth common mental disorders (Y-CMD), particularly developmental disorders, are warranted.

Interestingly, McGrath et al (2016)[14] found numerous significant associations between mental disorders and later PE. They have also found a sub-additive effect of comorbidity, supporting the lack of specificity of the association between PE and psychotic disorders. In adolescents, a 10-year community-based longitudinal study found bidirectional associations between mood symptoms and PE, with a dose-response effect for both directions.[23] However, this finding was not confirmed in help-seeking adolescents.[26] Thus, while several studies have investigated PE and later psychiatric disorders, few have addressed the opposite direction of this relationship, which may add to the question whether PE are specifically associated to full-blown psychotic syndrome. This is surprising given a number of reasons. *First*, there is solid evidence of high co-morbidity between Y-CMD – particularly depression – and psychotic disorders[27]; *second*, a large birth-cohort study showed shared risk factors between PE and depressive symptoms[28]; *third,* studies found shared genetic risk for psychotic and numerous non-psychotic disorders[29, 30]; *fourth*, help-seeking youth with non-psychotic psychiatric symptoms report high lifetime rates of PE[2]; *fifth*, population-based surveys found elevated rates of PE in depressive and anxiety disorders[31, 32] There is a need therefore to better understanding the bidirectional associations between PE and Y-CMD. This investigation seems well suited for samples focusing on early development since they are less confounded by treatment interventions and recall bias.

Findings from epidemiological samples showed that age is negatively correlated to PE.[1] Prevalence rates of PE are higher than 15% in childhood and early adolescence compared to 7% in adults.[33] This difference raises methodological concerns regarding how to best inquire PE during development, particularly for children under 10 years-old.[1, 34] Issues such as informant source (self- *vs* parent-report) and rating procedures (from highly trained clinicians to unguided self-report) are matter of considerable debate.[1, 33, 35] Possible ways to overcome these concerns, among

others, are using previously validated instruments[35], investigating both dimensional and categorical measures of PE, and confirm data from self-report with clinician rating.[1]

Here, we investigate the transdiagnostic associations between PE and Y-CMD in a longitudinal community-based sample. We addressed three aims: *first*, to investigate whether baseline PE predict Y-CMD: depression, anxiety, ADHD, and ODD/CD; *second*, to evaluate if Y-CMD are predictive of PE at follow-up; and *third*, to evaluate the bidirectional relationship between the number of comorbid Y-CMD with PE. We conduct all analyses examining both categorical and self-reported measures of PE, from clinical rating and self-report. We hypothesized that *i.* there is a link between PE and depression, resembling findings in late adolescence and early adulthood[10], and *ii.* Y-CMD comorbidity will significantly correlate to PE, showing a nonspecific effect of PE as a risk for a broad "psychiatric load/liability".

4.3.3 Methods

Sample - High-risk cohort (HRC) study

This study is part of a large school-based study that screened 9937 students from public schools of two large metropolitan areas in Brazil (for details see Salum et al (2015)[36]). Institutional Review Board of all involved universities provided ethical approval for the protocol. Parents provided written informed consent for all participants. Children provided verbal informed assent. Children who were able to read and write also provided written consent.

At screening phase, we invited parents from children aged 6-12 years old during school registry day to participate. Brazilian law obliges parents to registry their children in schools close to their home address. Parents were interviewed about psychiatric symptoms using the Family History Screen (FHS) (biological mother in 88% of the families).[37] From this pool, we evaluated 2511 subjects selected using two different criteria: a random stratum (n=958) and a high-risk stratum based on the family load of psychiatric symptoms from the FHS (n=1553). Around 3 years later, we invited subjects to participate in a follow-up household evaluation. We were able to re-evaluate 80.05% of the baseline sample. Main reasons for attrition at 3-year follow-up were refusal (10%) and loss of contact (10%). Children who met criteria for any anxiety disorder at baseline ($p<.01$) were at higher chance to successfully complete the follow-up, whereas lower maternal education ($p<.001$), lower socioeconomic status ($p<.05$), and

living in Porto Alegre City (p<.05) diminished the probability to attend to the follow-up evaluation. (Pan et al, accepted for publication)

Measures

Youth Common Mental Disorders (Y-CMD)

We used the Development and Well-Being Assessment (DAWBA) to assess DSM psychiatric disorders.[38] DAWBA is a validated structured interview developed to be administered by trained lay interviewers. The Brazilian Portuguese version shows appropriate psychometric proprieties and high inter-rater reliability.[36, 39] At baseline, we only assessed data on DAWBA psychopathology from parent-report since self-report may not me reliable for children younger than 11 years-old.[40] At follow-up, youth were interviewed by certified clinical psychologists and reported on DAWBA for emotional disorders. Trained lay interviewers performed the DAWBA interview with parents or the main caregiver of the adolescent. Psychiatrist raters evaluated parent- and self-reported DAWBA interview information to ascertain DSM-IV psychiatric diagnoses. They used a digital platform (*www.dawbanet.com*) to integrate both sources of information and to evaluate verbatim responses to open-ended questions. We trained raters to strictly follow DSM criteria. Inconclusive cases were discussed in regular meetings with senior psychiatrists from the research team. At follow-up, raters were blinded for baseline psychiatric disorders.

We created 4 categorical groups of Y-CMD according to DSM criteria for the present analysis: any Depressive Disorder (MDD, Other Specified Depressive Disorder and Unspecified Depressive Disorder), any Anxiety Disorder (Separation anxiety, Specific Phobia, Social phobia, Panic disorder, Agoraphobia, Post-traumatic Stress Disorder, Obsessive Compulsive Disorder, Generalized Anxiety Disorder, and Other Specified Anxiety), Any ODD/CD (ODD, CD, and Other Specified Disruptive Disorder), Any ADHD (ADHD combined, ADHD inattentive, ADHD hyperactive-impulsive, ADHD not otherwise specified (NOS)).

Dimensional PE

We used the 20-item positive scale from *Community Assessment of Psychotic Experiences (*CAPE) to assess PE in children and adolescents. Since we included children as young as 6 years-old at baseline, we trained certified psychologists to read the 20 items about positive symptoms to avoid literacy levels interfering with self-report. The positive scale from CAPE inquiries about several types of PE including auditory

hallucinations, passivity experiences, thought insertion, thought broadcasting, and delusional perception. Children rated *yes/no* to each item.[35, 41] For baseline and follow-up, Dimensional PE data were evaluable for 2,224 (89.4%) and 1877 (74.8%) respectively.

We performed psychometric evaluation for CAPE as a dimensional measure of self-report PE using Multilevel Confirmatory Factor Analysis for baseline and follow-up assessments using each psychologist as a level in the analysis. Models were adjusted for our oversampling design by using weighting procedures. We generated factor scores for each participant for both time points. All goodness-of-fit measures for models were in the acceptable range, as reported in Table S1.

Categorical construct of PE (CAARMS-PE)

We have evaluated PE as a categorical measure using *Comprehensive Assessment of At-risk Mental States* (CAARMS) rating questions.[42, 43] After rating CAPE, psychologists used the previously investigated information on PE to rate three CAARMS positive domains questions: unusual mental contents (delusional mood, overvalued ideas and delusions), unusual perceptual experiences (perceptual distortions, illusions and hallucinations), and speech/thought conceptual disorganization (objectively assessed by the psychologist according to speech and formal thought disorder anchors). Scores ranged from 0 to 6 according to CAARMS anchoring.[43] Anchors were available in all protocols and varied for each domain, with contextual examples of possible rating for each level.

We have operationalized a positive PE whenever CAARMS score was higher than 1 (Questionable) for any of the three domains. It means, for instance, that rating for unusual mental contents had to be higher or equal as a “vague sense that something is different, or not quite right with the world, a sense that things have changed but not able to be clearly articulated”, whereas a Questionable rating was a “mild elaboration of conventional beliefs as held by a proportion of the population”.

Categorical (CAARMS-PE) and dimensional (CAPE) PE data were evaluable for 1877 (74.8%) at follow-up. At both time points, data were evaluable for 1712 (76.3%) subjects. This attrition rate is higher than reported above(Pan et al, accepted for publication) because it only considered subjects with complete data from 4 assessments: baseline *i.* household parental interview and *ii.* scholar child evaluation, and follow-up *iii.* household parental and *iv.* household adolescent report interview.

Gender, age, and study site were not significantly different among subjects who completed and not completed the 4 assessment visits.

Statistical Analysis

Analyses were two-tailed and significance threshold was set at 5%. We used t-tests and chi-square tests to examine associations of gender, age, and study site and CAARMS-PE at both time points. This set of demographic analyses and reported prevalence rates for CAARMS-PE were weighted for HRC enrollment procedure.[44]

PE at baseline predicting Y-CMD at follow-up

Logistic regression models assessed the relationship of baseline CAARMS-PE with follow-up Y-CMD (outcome) in bivariate models, i.e. one model for each Y-CMD group: any Depressive Disorder, any Anxiety Disorder, any ODD/CD, and any ADHD. Then, in multivariate models, we also included all baseline Y-CMD as control variables.

We have also used logistic regression to test the associations of baseline dimensional PE and follow-up Y-CMD (outcome); again, we first ran bivariate models for each disorder and then multiple models including all baseline Y-CMD.

Y-CMD at baseline predicting PE at follow-up

Logistic regression models assessed the relationship of baseline Y-CMD and follow-up categorical PE (outcome). In bivariate models, we tested 4 models using baseline Y-CMD groups (any Depressive Disorder, any Anxiety Disorder, any ODD/CD, and any ADHD) as the binary outcome, and follow-up CAARMS-PE as the predictor. We then ran multivariate models adding all follow-up Y-CMD groups as control variables.

Logistic regression models tested the associations of baseline Y-CMD (outcome) and follow-up dimensional PE; again, we first ran bivariate models for each Y-CMD group and then multivariable models including all follow-up Y-CMD as nuisance predictors.

Comorbidity Analysis

We investigated the bidirectional effect of comorbid Y-CMD in PE. First, we created count variables for comorbid psychiatric disorders at baseline and follow-up (Figure S1). Poisson regression models tested the effect of PE (predictor) in the count variable (outcome), which represents the number of psychiatric comorbid disorders. Bivariate models included gender, age, and study site as nuisance variables. In multivariable models, we tested if this effect was significant when controlling for the co-

occurrence of Y-CMD and PE at the same time point. Therefore, Poisson regression models included PE and Y-CMD groups at the same time point as predictors and the number of comorbid disorders at the opposite time point as outcome.

4.3.4 Results

CAARMS-PE weighted rates at baseline and 3-year follow-up were 16.0% and 21.2%, respectively. There were no conversions to a psychotic disorder according to our diagnostic interview. Positive rating at baseline CAARMS-PE was significantly associated to female gender (OR 1.48, 95%CI= 1.10-1.99, p<.01) and younger age (t=4.84, d.f. 1404, p<.001). Follow-up CAARMS-PE positive rating was not significantly associated to gender (OR 0.81, 95%CI= 0.62-1.04, p=.10) and age (t=1.90, d.f. 1407, p=.06), even though there was a trend for older age in the positive CAARMS-PE group. Study site was significantly related to CAARMS-PE at follow-up (OR 1.84, 95%CI=1.41-2.42, p<.001). Therefore, herein, all analyses were controlled for sex, age, and study site.

PE at baseline and Y-CMD at follow-up

Baseline CAARMS-PE (OR 1.83, 95%CI 1.17-2.86, p<.01) and dimensional PE (OR 2.48, 95%CI1.55-3.97, p<.001) increased the likelihood of any depressive disorder at follow-up in bivariate models. Even after controlling for baseline Y-CMD, both CAARMS-PE (OR 1.77, 95%CI 1.12-2.79, p<.01) and dimensional self-report PE (OR 2.47, 95%CI 1.53-6.05, p<.001) predicted any depressive disorder at follow-up (Table 1).

Y-CMD at baseline and PE at follow-up

Table 2 shows findings from regression models evaluating baseline Y-CMD and subsequent PE. We found several significant associations in bivariate analyses: CAARMS-PE with any ODD/CD and any ADHD, dimensional PE with any anxiety, any ODD/CD and any ADHD. However, after adjusting for follow-up Y-CMD, only CAARMS-PE was significantly associated to any ADHD (OR 1.88, 95%CI 1.32-2.69, p<.001).

Comorbidity Analyses

In bivariate models, baseline CAARMS-PE (OR 1.41, 95%CI 1.16-1.73, p<.001) and dimensional PE (OR 1.32, 95%CI 1.08-1.61, p=.007) were significantly associated with number of comorbid Y-CMD at follow-up. After controlling for baseline Y-CMD

groups, dimensional PE remained a significant predictor (OR 1.29, 95%CI 1.06-1.56, p=.012), while CAARMS-PE did not (OR 1.19, 95%CI 0.97-1.46, p=.090).

Table 4.3.1 Psychotic Experiences at Baseline and Youth Common Mental Disorders at 3-year Follow-up

Categorical Psychotic Experiences (CAARMS)	**Bivariate models[1]**	**Multivariate Models[2]**
	OR (95% CI)	OR (95% CI)
Any Depressive Disorder	**1.83 (1.17-2.86)***	**1.77 (1.12-2.79)***
Any Anxiety Disorder	1.28 (0.90-1.83)	1.17 (0.81-1.70)
Any ODD/CD	1.19 (0.68-2.09)	0.97 (0.53-1.76)
Any ADHD	0.90 (0.49-1.66)	0.72 (0.38-1.36)
Dimensional Psychotic Experiences (CAPE)	**Bivariate models[1]**	**Multivariate Models[2]**
	OR (95% CI)	OR (95% CI)
Any Depressive Disorder	**2.48 (1.55-3.97)****	**2.47 (1.53-6.05)****
Any Anxiety Disorder	1.39 (0.98-1.96)	1.33 (0.93-1.90)
Any ODD/CD	1.09 (0.64-1.88)	0.90 (0.51-1.57)
Any ADHD	0.95 (0.54-1.66)	0.78 (0.44-1.39)

[1] Controlled for gender, site, age; [2] Controlled for previous confounders plus baseline common mental disorders;*p<.01, **p<.001. CAARMS, Comprehensive Assessment of At-risk Mental States; CAPE, Community Assessment of Psychotic Experiences.

We have also investigated the opposite direction of the relationship between comorbid Y-CMD and PE. We found, in bivariate models, that baseline comorbid Y-CMD were significantly associated to both CAARMS-PE (OR 1.61, 95%CI 1.30-1.98, p<.001) and dimensional PE (OR 1.60, 95%CI 1.34-1.92, p<.001) at follow-up. Lastly, multivariate models controlling for follow-up Y-CMD confirmed the bivariate finding for CAARMS-PE (OR 1.22, 95%CI 1.01-1.47, p<.038), whereas dimensional PE was not significantly associated in the multivariate model (OR 1.11, 95%CI 0.89-1.39, p=.368).

4.3.5 Discussion

The present study investigated the bidirectional associations between psychiatric disorders in youth and subclinical psychotic symptoms, commonly referred to as PE, in the transition from childhood to adolescence. To our knowledge, no previous longitudinal community-based study investigated comprehensively this

relationship merging self-report and clinical rating in this age range. PE at baseline increased the likelihood of any depressive disorder at follow-up. This finding was consistent through self-report and clinical rating, even after adjusting for baseline Y-CMD. When we explored baseline mental disorders and later PE, baseline anxiety, ADHD, and ODD/CD were significantly associated to PE 3 years later. After controlling for follow-up Y-CMD, ADHD remained significantly associated with PE. This finding however only emerged using CAARMS-PE – our clinician rated PE measure. Finally, number of comorbid psychiatric disorder was significantly related to higher PE in both comorbidity analyses, which showed that this feature is bidirectional. Therefore, we identified a nonspecific relationship between PE and comorbid psychiatric disorders, which suggests PE and common psychiatric disorders in youth share similar sources of overall susceptibility".

Table 4.3.2 Youth Common Mental Disorders at Baseline and Psychotic Experiences at 3-year Follow-up

Categorical - presence of PE in CAARMS	**Bivariate models[1]**	**Multivariate Models[2]**
	OR (95% CI)	OR (95% CI)
Any Depressive Disorder	1.65 (0.89-3.06)	1.21 (0.63-2.33)
Any Anxiety Disorder	1.35 (0.95-1.93)	1.05 (0.72-1.53)
Any ODD/CD	**1.81 (1.18-2.77)****	1.22 (0.76-1.95)
Any ADHD	**2.29 (1.64-3.17)*****	**1.88 (1.32-2.69)*****
Dimensional Psychotic Experiences – CAPE	**Bivariate models[1]**	**Multivariate Model[2]**
	OR (95% CI)	OR (95% CI)
Any Depressive Disorder	1.57 (0.74-3.32)	0.96 (0.43-2.14)
Any Anxiety Disorder	**1.59 (1.06-2.39)***	1.13 (0.74-1.74)
Any ODD/CD	**1.78 (1.07-2.97)****	1.03 (0.58-1.82)
Any ADHD	**1.85 (1.23-2.78)****	1.42 (0.91-2.22)

[1] Controlled for gender, site, age;[2] Controlled for previous confounders plus follow-up youth common mental disorders; *p<.05, **p<.01,***p<.001. CAARMS, Comprehensive Assessment of At-risk Mental States; CAPE, Community Assessment of Psychotic Experiences.

PE predicted later depressive disorders irrespective of baseline Y-CMD, which also included controlling for baseline depression. This finding confirmed previous results from cross-sectional and longitudinal studies.[10, 13, 17] Even though a study

found that clinical high-risk for psychosis syndrome – which requires more severe and impairing PE – was not predictive of mood and anxiety disorders[45], PE may be causally related to depression.[23] We hypothesize four causal pathways to explain this relationship. *First*, PE might be early symptoms of depressive disorder.[16] *Second*, PE might be manifestations of early biological mechanisms implicated in the pathogenesis of depressive disorder.[13] *Third*, PE and sub-threshold mood symptoms dynamically interact with each other increasing the risk of later non-psychotic disorder given the level of environmental exposure.[46] *Forth*, PE are perceived as traumatic life events, which in turn increase the likelihood of developing a depressive disorder. *Finally*, some specific dimensions of PE are a developmental early manifestation or may confer risk for nonspecific psychopathology.[47] Future studies investigating biological markers related to each disorder, such as polygenic risk scores, may elucidate the underlying shared liability of PE and mood disorders. Clinicians should pay attention for the emergence of mood symptoms in youth presenting PE since they may be an early marker of – or putatively play a causal role for – depressive disorder.

Clinical manifestations of ADHD symptoms may be an early extended phenotype of risk for psychosis. Indeed, several studies found inattentive symptoms in subjects at risk for psychosis.[48] Using the basic symptom-based criterion, Ruhrmann et al (2010)[49] showed the relevance of cognitive disturbances in the putatively prodromal state of psychosis. Among cognitive early symptoms, there were inattentive symptoms such as inability to divide attention; moreover, their recruitment included referrals for concentration and attention disturbances.[49] However, our ADHD finding contrasts with a recent report from the Multimodal Treatment Study of Children with ADHD study showing similar levels of PE in ADHD patients and controls. PE were frequently transient, prevalence was low (around 5%), and few cases had PE confirmed by a clinician.[25] Future research on this topic should focus on disentangling the effect of inattentive cognitive symptoms as a developmental early correlate or a heterotypic precursor of the psychotic phenotype.

Our comorbidity analyses are in line with findings from McGrath et al (2016)[14] showing a nonspecific association between PE and several psychiatric disorders. Moreover, in bivariate models (i.e. not adjusted for psychopathology), we found significant associations of temporally primary anxiety, ODD/CD, and ADHD with subsequent PE. This suggests that PE is a nonspecific marker of severity or a broader marker of “psychiatric load/liability”. The role of PE has been the focus of an interesting

debate in the recent years, which has implications for research and clinical practice.[9, 15, 50-52] Similar results showing the lack of specificity of PE to predict psychotic disorder have even been discussed as possible evidence for re-evaluating the taxonomy of psychotic symptoms in diagnostic manuals.[50] Whether PE specifically predict psychotic disorders as a function of distress and severity, as postulated in ultra-high risk research, or account for a nonspecific "transphenotypic"[15] liability to mental disorders remains uncertain. In the general population, such as our community-based sample, the later hypothesis seems reasonable and is supported by our findings.

Our study has limitations. The attrition rate of approximately 25% may have biased our sample loosing possible cases with higher PE or psychiatric morbidity. In addition, attrition may have limited our statistical power to show small effect-size relationships. This seemed particularly important for analyses of PE as precursors of subsequent PE. However, losses at follow-up are expected in community-based studies, and the prevalence of psychiatric disorders at both time-points were similar. We cannot exclude reverse causality, especially in bivariate analysis showing nonspecific associations of Y-CMD and subsequent PE, even though our longitudinal design diminished reporting bias expected in cross-sectional studies. We partially addressed this issue by adjusting models for the presence of baseline Y-CMD. By doing so, we control for the possibility that prediction for depressive disorder was a by-product of the co-occurrence of PE and depression at baseline. Our study also have strengths. We confirmed previously reported cross-sectional results of the nonspecific association between PE and Y-CMD using a longitudinal design. Moreover, we assessed PE exploring and comparing self-report dimensional measures and clinical categorical rating. To our knowledge, no previous study investigated the association of PE and Y-CMD using this comprehensive approach.

We have demonstrated that PE predicted subsequent depressive disorder; we have also identified nonspecific relationships between temporally primary mental disorders and later PE in a longitudinal sample of late childhood and early adolescence. In comorbidity analyses, this pattern of nonspecific associations were confirmed. Since both phenomena are quite prevalent in this age range, further studies may incorporate biological markers to investigate potential underlying pathophysiological mechanisms for their relationship. For now, clinicians should be aware of the possibility that PE are clinical manifestations that predict – and may be predicted by – mental disorders. These findings may inform future research on testing

subclinical psychotic symptoms to further our understating on identifying high-risk groups for early intervention.

4.3.6 References

1. Linscott RJ, van Os J. An updated and conservative systematic review and meta-analysis of epidemiological evidence on psychotic experiences in children and adults: on the pathway from proneness to persistence to dimensional expression across mental disorders. Psychological medicine. 2013;43(6):1133-49.

2. Yung AR, Buckby JA, Cotton SM, Cosgrave EM, Killackey EJ, Stanford C, et al. Psychotic-like experiences in nonpsychotic help-seekers: associations with distress, depression, and disability. Schizophrenia bulletin. 2006;32(2):352-9.

3. Honings S, Drukker M, Groen R, van Os J. Psychotic experiences and risk of self-injurious behaviour in the general population: a systematic review and meta-analysis. Psychological medicine. 2016;46(2):237-51.

4. Polanczyk G, Moffitt TE, Arseneault L, Cannon M, Ambler A, Keefe RS, et al. Etiological and clinical features of childhood psychotic symptoms: results from a birth cohort. Archives of general psychiatry. 2010;67(4):328-38.

5. Honings S, Drucker M, Ten Have M, de Graaf R, van Dorsselaer S, van Os J. Psychotic Experiences and Risk of Violence Perpetration and Arrest in the General Population: A Prospective Study. PloS one. 2016;11(7):e0159023.

6. Bhavsar V, Maccabe JH, Hatch SL, Hotopf M, Boydell J, McGuire P. Subclinical psychotic experiences and subsequent contact with mental health services. BJPsych Open. 2017;3(2):64-70.

7. Sharifi V, Eaton WW, Wu LT, Roth KB, Burchett BM, Mojtabai R. Psychotic experiences and risk of death in the general population: 24-27 year follow-up of the Epidemiologic Catchment Area study. The British journal of psychiatry : the journal of mental science. 2015;207(1):30-6.

8. Fisher HL, Caspi A, Poulton R, Meier MH, Houts R, Harrington H, et al. Specificity of childhood psychotic symptoms for predicting schizophrenia by 38 years of age: a birth cohort study. Psychological medicine. 2013;43(10):2077-86.

9. van Os J, Guloksuz S. A critique of the "ultra-high risk" and "transition" paradigm. World psychiatry : official journal of the World Psychiatric Association. 2017;16(2):200-6.

10. Kaymaz N, Drukker M, Lieb R, Wittchen HU, Werbeloff N, Weiser M, et al. Do subthreshold psychotic experiences predict clinical outcomes in unselected non-help-seeking population-based samples? A systematic review and meta-analysis, enriched with new results. Psychological medicine. 2012;42(11):2239-53.

11. Poulton R, Caspi A, Moffitt TE, Cannon M, Murray R, Harrington H. Children's self-reported psychotic symptoms and adult schizophreniform disorder: a 15-year longitudinal study. Archives of general psychiatry. 2000;57(11):1053-8.

12. Calkins ME, Moore TM, Satterthwaite TD, Wolf DH, Turetsky BI, Roalf DR, et al. Persistence of psychosis spectrum symptoms in the Philadelphia Neurodevelopmental Cohort: a prospective two-year follow-up. World psychiatry : official journal of the World Psychiatric Association. 2017;16(1):62-76.

13. Kelleher I, Keeley H, Corcoran P, Lynch F, Fitzpatrick C, Devlin N, et al. Clinicopathological significance of psychotic experiences in non-psychotic young people: evidence from four population-based studies. The British journal of psychiatry : the journal of mental science. 2012;201(1):26-32.

14. McGrath JJ, Saha S, Al-Hamzawi A, Andrade L, Benjet C, Bromet EJ, et al. The Bidirectional Associations Between Psychotic Experiences and DSM-IV Mental Disorders. The American journal of psychiatry. 2016;173(10):997-1006.

15. van Os J, Reininghaus U. Psychosis as a transdiagnostic and extended phenotype in the general population. World psychiatry : official journal of the World Psychiatric Association. 2016;15(2):118-24.

16. Armando M, Nelson B, Yung AR, Ross M, Birchwood M, Girardi P, et al. Psychotic-like experiences and correlation with distress and depressive symptoms in a community sample of adolescents and young adults. Schizophrenia research. 2010;119(1-3):258-65.

17. Wigman JT, van Nierop M, Vollebergh WA, Lieb R, Beesdo-Baum K, Wittchen HU, et al. Evidence that psychotic symptoms are prevalent in disorders of anxiety and depression, impacting on illness onset, risk, and severity--implications for diagnosis and ultra-high risk research. Schizophrenia bulletin. 2012;38(2):247-57.

18. Insel TR. Rethinking schizophrenia. Nature. 2010;468(7321):187-93.

19. Collins PY, Patel V, Joestl SS, March D, Insel TR, Daar AS, et al. Grand challenges in global mental health. Nature. 2011;475(7354):27-30.

20. Keshavan MS, Diwadkar VA, Montrose DM, Rajarethinam R, Sweeney JA. Premorbid indicators and risk for schizophrenia: a selective review and update. Schizophrenia research. 2005;79(1):45-57.

21. Paus T. Population neuroscience: why and how. Hum Brain Mapp. 2010;31(6):891-903.

22. Kim-Cohen J, Caspi A, Moffitt TE, Harrington H, Milne BJ, Poulton R. Prior juvenile diagnoses in adults with mental disorder: developmental follow-back of a prospective-longitudinal cohort. Archives of general psychiatry. 2003;60(7):709-17.

23. van Rossum I, Dominguez MD, Lieb R, Wittchen HU, van Os J. Affective dysregulation and reality distortion: a 10-year prospective study of their association and clinical relevance. Schizophrenia bulletin. 2011;37(3):561-71.

24. Werbeloff N, Drukker M, Dohrenwend BP, Levav I, Yoffe R, van Os J, et al. Self-reported attenuated psychotic symptoms as forerunners of severe mental disorders later in life. Archives of general psychiatry. 2012;69(5):467-75.

25. Vitiello B, Perez Algorta G, Arnold LE, Howard AL, Stehli A, Molina BS. Psychotic Symptoms in Attention-Deficit/Hyperactivity Disorder: An Analysis of the MTA Database. Journal of the American Academy of Child and Adolescent Psychiatry. 2017;56(4):336-43.

26. Wigman JT, Lin A, Vollebergh WA, van Os J, Raaijmakers QA, Nelson B, et al. Subclinical psychosis and depression: co-occurring phenomena that do not predict each other over time. Schizophrenia research. 2011;130(1-3):277-81.

27. Buckley PF, Miller BJ, Lehrer DS, Castle DJ. Psychiatric comorbidities and schizophrenia. Schizophrenia bulletin. 2009;35(2):383-402.

28. Kounali D, Zammit S, Wiles N, Sullivan S, Cannon M, Stochl J, et al. Common versus psychopathology-specific risk factors for psychotic experiences and depression during adolescence. Psychological medicine. 2014;44(12):2557-66.

29. Cross-Disorder Group of the Psychiatric Genomics C. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet. 2013;381(9875):1371-9.

30. Cross-Disorder Group of the Psychiatric Genomics C, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nature genetics. 2013;45(9):984-94.

31. Varghese D, Scott J, Welham J, Bor W, Najman J, O'Callaghan M, et al. Psychotic-like experiences in major depression and anxiety disorders: a population-based survey in young adults. Schizophrenia bulletin. 2011;37(2):389-93.

32. Morgan C, Reininghaus U, Reichenberg A, Frissa S, team SEs, Hotopf M, et al. Adversity, cannabis use and psychotic experiences: evidence of cumulative and synergistic effects. The British journal of psychiatry : the journal of mental science. 2014;204:346-53.

33. Kelleher I, Connor D, Clarke MC, Devlin N, Harley M, Cannon M. Prevalence of psychotic symptoms in childhood and adolescence: a systematic review and meta-analysis of population-based studies. Psychological medicine. 2012;42(9):1857-63.

34. Pontillo M, De Luca M, Pucciarini ML, Vicari S, Armando M. All that glitters is not gold: prevalence and relevance of psychotic-like experiences in clinical sample of children and adolescents aged 8-17 years old. Early intervention in psychiatry. 2016.

35. Konings M, Bak M, Hanssen M, van Os J, Krabbendam L. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta psychiatrica Scandinavica. 2006;114(1):55-61.

36. Salum GA, Gadelha A, Pan PM, Moriyama TS, Graeff-Martins AS, Tamanaha AC, et al. High risk cohort study for psychiatric disorders in childhood: rationale, design, methods and preliminary results. International journal of methods in psychiatric research. 2015;24(1):58-73.

37. Weissman MM, Wickramaratne P, Adams P, Wolk S, Verdeli H, Olfson M. Brief screening for family psychiatric history: the family history screen. Archives of general psychiatry. 2000;57(7):675-82.

38. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. Journal of child psychology and psychiatry, and allied disciplines. 2000;41(5):645-55.

39. Fleitlich-Bilyk B, Goodman R. Prevalence of child and adolescent psychiatric disorders in southeast Brazil. Journal of the American Academy of Child and Adolescent Psychiatry. 2004;43(6):727-34.

40. Schwab-Stone M, Fallon T, Briggs M, Crowther B. Reliability of diagnostic reporting for children aged 6-11 years: a test-retest study of the Diagnostic Interview Schedule for Children-Revised. The American journal of psychiatry. 1994;151(7):1048-54.

41. Mark W, Toulopoulou T. Psychometric Properties of "Community Assessment of Psychic Experiences": Review and Meta-analyses. Schizophrenia bulletin. 2016;42(1):34-44.

42. Yung AR, Phillips LJ, Yuen HP, Francey SM, McFarlane CA, Hallgren M, et al. Psychosis prediction: 12-month follow up of a high-risk ("prodromal") group. Schizophrenia research. 2003;60(1):21-32.

43. Yung AR, Yuen HP, McGorry PD, Phillips LJ, Kelly D, Dell'Olio M, et al. Mapping the onset of psychosis: the Comprehensive Assessment of At-Risk Mental States. The Australian and New Zealand journal of psychiatry. 2005;39(11-12):964-71.

44. Hoffmann MS, Leibenluft E, Stringaris A, Laporte PP, Pan PM, Gadelha A, et al. Positive Attributes Buffer the Negative Associations Between Low Intelligence and High Psychopathology With Educational Outcomes. Journal of the American Academy of Child and Adolescent Psychiatry. 2016;55(1):47-53.

45. Webb JR, Addington J, Perkins DO, Bearden CE, Cadenhead KS, Cannon TD, et al. Specificity of Incident Diagnostic Outcomes in Patients at Clinical High Risk for Psychosis. Schizophrenia bulletin. 2015;41(5):1066-75.

46. Guloksuz S, van Nierop M, Lieb R, van Winkel R, Wittchen HU, van Os J. Evidence that the presence of psychosis in non-psychotic disorder is environment-dependent and mediated by severity of non-psychotic psychopathology. Psychological medicine. 2015;45(11):2389-401.

47. Yung AR, Nelson B, Baker K, Buckby JA, Baksheev G, Cosgrave EM. Psychotic-like experiences in a community sample of adolescents: implications for the continuum model of psychosis and prediction of schizophrenia. The Australian and New Zealand journal of psychiatry. 2009;43(2):118-28.

48. Schultze-Lutter F, Klosterkotter J, Ruhrmann S. Improving the clinical prediction of psychosis by combining ultra-high risk criteria and cognitive basic symptoms. Schizophrenia research. 2014;154(1-3):100-6.

49. Ruhrmann S, Schultze-Lutter F, Salokangas RK, Heinimaa M, Linszen D, Dingemans P, et al. Prediction of psychosis in adolescents and young adults at high

risk: results from the prospective European prediction of psychosis study. Archives of general psychiatry. 2010;67(3):241-51.

50. Kelleher I, Cannon M. Putting Psychosis in Its Place. The American journal of psychiatry. 2016;173(10):951-2.

51. Fusar-Poli P, Schultze-Lutter F, Cappucciati M, Rutigliano G, Bonoldi I, Stahl D, et al. The Dark Side of the Moon: Meta-analytical Impact of Recruitment Strategies on Risk Enrichment in the Clinical High Risk State for Psychosis. Schizophrenia bulletin. 2016;42(3):732-43.

52. Yung AR, Lin A. Psychotic experiences and their significance. World psychiatry : official journal of the World Psychiatric Association. 2016;15(2):130-1.

4.3.7 Supplemental Material

Table 4.3.S1 – Model fit for dimensional models of psychotic symptoms.

	RMSEA (90% CI)	CFI	TLI	WRMR	Chi-square
Baseline					
CAPE 20 items self-report	0.015 (0.011 0.019)	0.945	0.939	1.292	255.394
3-year Follow up					
CAPE 20 items self-report	0.014 (0.009 0.018)	0.910	0.899	1.176	234.165

CAPE, Community Assessment of Psychotic Experiences; RMSEA, root mean square error of approximation; CFI, comparative fit index; TLI, Tucker Lewis index; WRMR, weighted root-mean-square residual

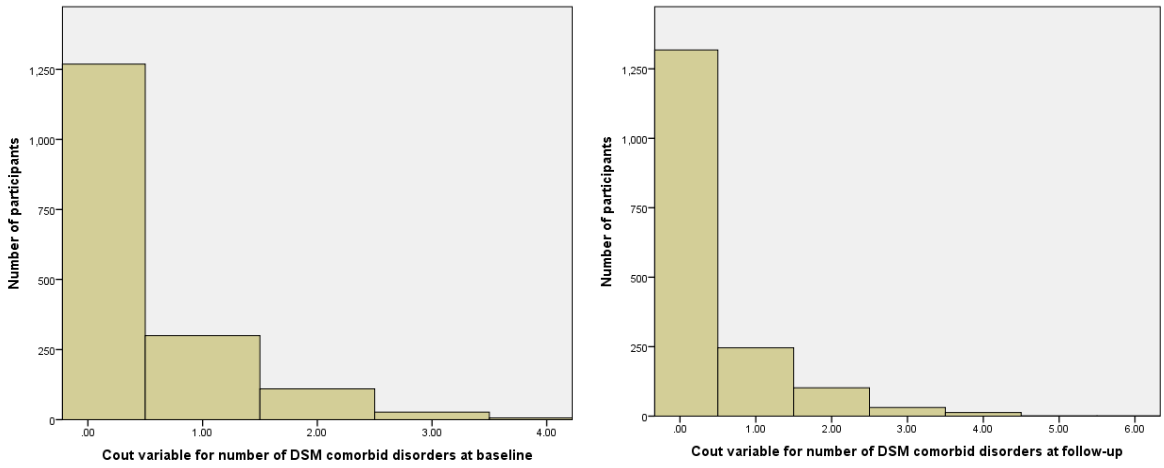

Figure 4.3.S1 Distribution for Variable Number of Comorbid Youth-Common Mental Disorders at baseline (left) and 3-year follow-up (right)

5. CONCLUSIONS

5.1 Main

We found that early developmental psychopathological factors (psychotic experiences), altered expression of inflammatory and neurodevelopmental genes, and functional connectivity of the reward brain circuitry were associated with mood disorders at the transition from childhood to adolescence.

5.2 Specifics

5.2.1 Study 1

- *NR3C1*, *TNF*, *TNFR1* and *IL1B* expression levels were associated with depressive disorder
- Expression of this set of genes jointly mediated the effect of childhood maltreatment on the risk of developing depressive disorder.
- Peripheral expression of genes implicated in the inflammation cascade partially explained the well-known association between childhood maltreatment and depressive disorder.
- The role of glucocorticoids as potential mediators for environmental stress in child and adolescent depressive disorder was confirmed.

5.2.2 Study 2

- Increased reward circuitry connectivity in 6-12 years old subjects predicted depressive disorder after 3 years.
- Reward processing has been postulated as an important mechanism in the pathogenesis of depressive disorders and may be used as an early risk marker of depression.[70, 71]

5.2.3 Study 3

- Psychotic experiences predicted depressive disorder after 3 years, irrespective of meeting criteria for depressive disorder at baseline.

- ADHD at baseline was significantly associated with psychotic experiences at follow-up, i.e. ADHD predicted psychotic experiences even after controlling for baseline common mental disorders.
- Greater numbers of comorbid mental disorders at baseline increased the odds of presenting psychotic experiences at follow-up
- Psychotic experiences at baseline also predicted the number of comorbid mental disorders at follow-up.

5.3 Final Conclusions

We found that some specific psychopathological, genetic and neuroimaging factors were related to depressive disorder during neurodevelopment. If replicated, our findings point toward more specific targets for identifying individuals who are at risk of depression and help in designing future treatment interventions to prevent depression among children and adolescents.

6. CONSIDERAÇÕES FINAIS

Este capítulo foi incorporado à tese após a realização da defesa pública, a partir de sugestões dos membros da banca de avaliação. O objetivo das próximas seções é discutir de forma mais abrangente as implicações dos resultados apresentados para o campo de pesquisa.

6.1 Impacto dos Resultados para o Campo de Pesquisa

Os resultados encontrados nos estudos que compõe a tese somam-se aos achados prévios que apontam para o papel do neurodesenvolvimento atípico na etiopatogenia dos transtornos de humor. No Estudo 1 foi possível demonstrar o papel da expressão de genes que regulam a resposta ao estresse e à inflamação como mediadores da relação entre trauma precoce e depressão. Nesse estudo foi utilizado um modelo estatístico de mediação, cujo principal atributo é “injetar” causalidade e elucidar trajetórias para fatores de risco, mecanismos e desfechos clínicos.[7, 72, 73]

Dentre os 4 genes que apresentaram menor expressão no grupo de sujeitos com depressão destaca-se o gene NC3R1, responsável pela expressão de um grupo de receptores de glicocorticoides na membrana celular. Esses receptores foram implicados em estudos epigenéticos com modelos animais e atuam como mediadores do efeito do meio ambiente de proteção (cuidado materno) na ativação de determinados comportamentos.[74]

Entender as vias moleculares que ligam o ambiente a um determinado fenótipo de interesse tem o potencial de abrir novos caminhos para intervenções medicamentosas com alvos epigenéticos, como a expressão ou a metilação, conforme sugere Szyf (2017).[75] Pode, também, mudar a forma de entender o mecanismo de ação de intervenções já estabelecidas, como a psicoterapia, que poderia ser compreendida como uma forma de “medicação epigenética”.[76] Todavia, é improvável que a expressão de um único gene consiga explicar toda a variabilidade interindividual encontrada no transtorno depressivo. De fato, entende-se atualmente que o componente genético da depressão é poligênico e inespecífico, o que já foi demonstrado nos estudos de GWAS.[10, 77]

O resultado encontrado no modelo de mediação do Estudo 1 indica justamente que a soma da expressão de genes de mecanismos parcialmente correlatos, em vez

da expressão isolada de cada gene, foi um mediador estatisticamente significante da associação entre trauma precoce e depressão. Assim, sugere-se que o trauma precoce, de maneira análoga ao padrão de risco encontrado nos estudos do genótipo, tem um pequeno efeito sobre a expressão de diversos genes. O maior risco para o desenvolvimento de transtornos de humor ocorreria, contudo, somente por meio da soma de várias alterações epigenéticas. Essa hipótese aponta para a necessidade de estudos futuros utilizarem técnicas de escaneamento da expressão do maior número possível de genes, assim como nos estudos de GWAS, chamados de estudo de transcriptoma. Apesar de substancialmente mais caros, essa metodologia permite a investigação de milhares de genes em uma única amostra. Além disso, não necessita de hipóteses *a priori,* como no Estudo 1, no qual se escolheu determinados genes através de pesquisa da literatura.

Pode-se traçar um paralelo entre os achados genéticos do Estudo 1 e os resultados psicopatológicos do Estudo 3. Ambos apontam para o efeito inespecífico de um marcador precoce de risco, seja biológico ou clínico. Assim como a soma da expressão de diversos genes foi importante para o desenvolvimento de depressão, as experiências psicóticas subclínicas associaram-se, de forma cumulativa e inespecífica, com o número de transtornos mentais comórbidos. Dessa forma, podem ser consideradas marcadores de risco para diversos transtornos mentais, em vez de sinais patognomônicos para o desenvolvimento de um determinado transtorno mental. Essa constatação tem implicações práticas, pois suporta recentes questionamentos sobre a identificação de jovens em alto risco para psicose exclusivamente por meio de sintomas psicóticos.[30] Em vez disso, os resultados sugerem que as experiências psicóticas são marcadores psicopatológicos de risco para o desenvolvimento de diversos transtornos mentais. Uma hipótese interessante, a ser investigada em estudos futuros, é que as experiências psicóticas atuam como marcadores clínicos de risco, entretanto, de um risco mais grave se comparado a outros sintomas precoces menos “tóxicos”, como sintomas de ansiedade.

O Estudo 2 integrou a procura de mecanismos cerebrais implicados na etiologia do transtorno depressivo com a necessidade de resultados que possam ter implicações para a clínica. Há uma interessante discussão nessa área: de um lado, pesquisadores que investem seus esforços na busca mecanismos cerebrais diretamente implicados na etiopatogenia dos transtornos mentais[78], e do outro lado, pesquisadores que acreditam na necessidade de buscar biomarcadores com potencial

para guiar a prática clínica em primeiro lugar, independentemente de sua função específica para a etiologia.[79] Apesar do argumento deste grupo ser bastante atraente, a totalidade dos estudos realizados nas últimas décadas ainda não mudaram o diagnóstico psiquiátrico no exercício clínico diário.[80, 81] Em contrapartida, o entendimento de mecanismos cerebrais específicos para determinados transtornos psiquiátrico começa a apresentar resultados interessantes. A identificação de um padrão cerebral especifico de resposta a faces de ameaça nos transtornos de ansiedade, por exemplo, proporcionou o desenvolvimento de novas modalidades de tratamento.[82]

Os resultados encontrados no Estudo 2, apesar de promissores nas análises de grupo, ainda não permitem a identificação de indivíduos em risco para o transtorno depressivo. Consequentemente, não se pode considerar a alteração encontrada no circuito de recompensa de crianças que desenvolveram depressão na adolescência como um biomarcador. Essa conclusão é frequentemente encontrada em estudos de neuroimagem e constitui o principal desafio atual para o campo de pesquisa no momento.

Avançar o conhecimento acumulado sobre as diferenças encontradas em análises de grupo, comparando pacientes e controles saudáveis, ainda é um desafio. No futuro, espera-se que um exame de Ressonância Magnética possa prover informações úteis para o clínico sobre diagnóstico e tratamento de seu paciente. O desenvolvimento de técnicas matemáticas e de classificadores automatizados que buscam padrões, como *Machine Learning,* pode ser um dos caminhos para a neuroimagem transformar a prática psiquiátrica. Com efeito, um estudo recentemente publicado em adultos com depressão utilizou ressonância magnética funcional em repouso, a mesma utilizada no Estudo 2, na tentativa de encontrar grupos de pacientes com base em padrões ou “assinaturas” de ativação cerebral. Os autores identificaram grupos de pacientes, confirmados em uma amostra independente de validação, que além de valor preditivo para o diagnóstico, apresentavam diferentes respostas para o tratamento com estimulação magnética transcraniana.[83]

6.2 Direções Futuras

A equipe da Coorte de Alto Risco para o Desenvolvimento de Transtornos Mentais e Resiliência (*High Risk Cohort* – HRC) já planeja o início da terceira fase de coleta de dados, isto é, a avaliação de 6 anos de seguimento. Os sujeitos, então entre 12 e 20 anos de idade, serão novamente convidados a participar das avaliações. Uma terceira avaliação permitirá o avanço do estudo de fatores de risco para a identificação de trajetórias do neurodesenvolvimento, típicas e atípicas. Além de manter o foco na avaliação minuciosa de sintomas psicopatológicos, o foco da equipe será investigar desfechos positivos, associados com o desenvolvimento saudável, e fatores de proteção, capazes de anular ou mitigar fatores de risco para os transtornos mentais.

Novos métodos de análise devem ser utilizados para explorar o banco de dados na busca de padrões alterados de neurodesenvolvimento como fator etiológico para transtornos de humor, como técnicas de *Machine Learning,* análise de transição latente (*Latent Transition Analysis*) e modelos latentes de crescimento (*Latent Growth Models*). Os métodos citados podem facilitar a integração de informações de diferentes métodos ou fontes de variância, aumentando a capacidade de determinar mecanismos implicados na etiopatogenia de um determinado fenótipo de interesse.

As últimas décadas foram marcadas por consideráveis avanços da neurociência e da psiquiatria. Contudo, houve pouca tradução desse conhecimento para a prática diária em saúde mental. É pouco provável que, ao menos nos próximos anos, ocorram mudanças radicais na forma como identificamos e tratamos os transtornos mentais. Se as pesquisas conseguirem, entretanto, promover mudanças e informar decisões da prática clínica no sentido de uma medicina mais personalizada, tal qual ocorre há poucos anos na oncologia, por exemplo, o campo já terá avançado extraordinariamente.

7. REFERÊNCIAS

1. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet. 2013;382(9904):1575-86.
2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of general psychiatry. 2005;62(6):593-602.
3. Scott J, Fowler D, McGorry P, Birchwood M, Killackey E, Christensen H, et al. Adolescents and young adults who are not in employment, education, or training. BMJ. 2013;347:f5270.
4. Levitt PR, March JS. Transformative Neurodevelopmental Research in Mental Illness. http://www.nimh.nih.gov/: Report of the National Advisory Mental Health Council's Workgroup; 2006.
5. Insel TR, Quirion R. Psychiatry as a clinical neuroscience discipline. JAMA : the journal of the American Medical Association. 2005;294(17):2221-4.
6. Insel TR. Rethinking schizophrenia. Nature. 2010;468(7321):187-93.
7. Paus T. Population Neuroscience. Berlin Heidelberg: Springer-Verlag; 2013.
8. Burmeister M, McInnis MG, Zöllner S. Psychiatric genetics: progress amid controversy. Nature Reviews Genetics. 2008;9(7):527-40.
9. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. The American journal of psychiatry. 2000;157(10):1552-62.
10. Cross-Disorder Group of the Psychiatric Genomics C, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nature genetics. 2013;45(9):984-94.
11. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53.
12. van Os J, Kenis G, Rutten BP. The environment and schizophrenia. Nature. 2010;468(7321):203-12.
13. Keshavan MS, Diwadkar VA, Montrose DM, Rajarethinam R, Sweeney JA. Premorbid indicators and risk for schizophrenia: a selective review and update. Schizophrenia research. 2005;79(1):45-57.
14. Arseneault L, Cannon M, Fisher HL, Polanczyk G, Moffitt TE, Caspi A. Childhood trauma and children's emerging psychotic symptoms: A genetically sensitive longitudinal cohort study. The American journal of psychiatry. 2011;168(1):65-72.
15. Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, et al. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biological psychiatry. 2005;57(10):1117-27.
16. Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci. 2006;7(7):583-90.
17. Yang Z, Zuo XN, McMahon KL, Craddock RC, Kelly C, de Zubicaray GI, et al. Genetic and Environmental Contributions to Functional Connectivity Architecture of the Human Brain. Cerebral cortex. 2016;26(5):2341-52.
18. McGorry PD, Yung A, Phillips L. Ethics and early intervention in psychosis: keeping up the pace and staying in step. Schizophrenia research. 2001;51(1):17-29.
19. Yung AR, McGorry PD. The initial prodrome in psychosis: descriptive and qualitative aspects. The Australian and New Zealand journal of psychiatry. 1996;30(5):587-99.

7. REFERÊNCIAS

20. Phillips LJ, Yung AR, McGorry PD. Identification of young people at risk of psychosis: validation of Personal Assessment and Crisis Evaluation Clinic intake criteria. The Australian and New Zealand journal of psychiatry. 2000;34 Suppl:S164-9.
21. Yung AR, McGorry PD, McFarlane CA, Jackson HJ, Patton GC, Rakkar A. Monitoring and care of young people at incipient risk of psychosis. Schizophrenia bulletin. 1996;22(2):283-303.
22. Correll CU, Hauser M, Auther AM, Cornblatt BA. Research in people with psychosis risk syndrome: a review of the current evidence and future directions. Journal of child psychology and psychiatry, and allied disciplines. 2010;51(4):390-431.
23. McGorry PD, Nelson B, Amminger GP, Bechdolf A, Francey SM, Berger G, et al. Intervention in individuals at ultra-high risk for psychosis: a review and future directions. The Journal of clinical psychiatry. 2009;70(9):1206-12.
24. Amminger GP, Schafer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, et al. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Archives of general psychiatry. 2010;67(2):146-54.
25. Morrison AP, French P, Walford L, Lewis SW, Kilcommons A, Green J, et al. Cognitive therapy for the prevention of psychosis in people at ultra-high risk: randomised controlled trial. The British journal of psychiatry : the journal of mental science. 2004;185:291-7.
26. Ruhrmann S, Bechdolf A, Kuhn KU, Wagner M, Schultze-Lutter F, Janssen B, et al. Acute effects of treatment for prodromal symptoms for people putatively in a late initial prodromal state of psychosis. The British journal of psychiatry Supplement. 2007;51:s88-95.
27. McGorry PD, Yung AR, Phillips LJ, Yuen HP, Francey S, Cosgrave EM, et al. Randomized controlled trial of interventions designed to reduce the risk of progression to first-episode psychosis in a clinical sample with subthreshold symptoms. Archives of general psychiatry. 2002;59(10):921-8.
28. Morrison AP, French P, Stewart SL, Birchwood M, Fowler D, Gumley AI, et al. Early detection and intervention evaluation for people at risk of psychosis: multisite randomised controlled trial. BMJ. 2012;344:e2233.
29. McGorry PD, Nelson B, Markulev C, Yuen HP, Schafer MR, Mossaheb N, et al. Effect of omega-3 Polyunsaturated Fatty Acids in Young People at Ultrahigh Risk for Psychotic Disorders: The NEURAPRO Randomized Clinical Trial. JAMA Psychiatry. 2017;74(1):19-27.
30. van Os J, Guloksuz S. A critique of the "ultra-high risk" and "transition" paradigm. World psychiatry : official journal of the World Psychiatric Association. 2017;16(2):200-6.
31. Thompson KN, Conus PO, Ward JL, Phillips LJ, Koutsogiannis J, Leicester S, et al. The initial prodrome to bipolar affective disorder: prospective case studies. Journal of affective disorders. 2003;77(1):79-85.
32. Correll CU, Penzner JB, Frederickson AM, Richter JJ, Auther AM, Smith CW, et al. Differentiation in the preonset phases of schizophrenia and mood disorders: evidence in support of a bipolar mania prodrome. Schizophrenia bulletin. 2007;33(3):703-14.
33. Conus P, Ward J, Lucas N, Cotton S, Yung AR, Berk M, et al. Characterisation of the prodrome to a first episode of psychotic mania: results of a retrospective study. Journal of affective disorders. 2010;124(3):341-5.
34. Fletcher RH, Fletcher SW. Clinical epidemiology : the essentials. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. xv, 252 p. p.
35. Schneider MR, DelBello MP, McNamara RK, Strakowski SM, Adler CM. Neuroprogression in bipolar disorder. Bipolar disorders. 2012;14(4):356-74.
36. McGorry PD, Killackey E, Yung A. Early intervention in psychosis: concepts, evidence and future directions. World psychiatry : official journal of the World Psychiatric Association. 2008;7(3):148-56.

7. REFERÊNCIAS

37. Geiger AM, Yu O, Herrinton LJ, Barlow WE, Harris EL, Rolnick S, et al. A population-based study of bilateral prophylactic mastectomy efficacy in women at elevated risk for breast cancer in community practices. Arch Intern Med. 2005;165(5):516-20.
38. Vieta E. Staging and psychosocial early intervention in bipolar disorder. Lancet Psychiatry. 2015;2(6):483-5.
39. Paus T. Population neuroscience: why and how. Hum Brain Mapp. 2010;31(6):891-903.
40. Hill AB. The environment and disease: association or causation? (Reprinted from JRSM, vol 58, 1965). J Roy Soc Med. 2015;108(1):32-7.
41. Duffy A, Carlson GA. How does a Developmental Perspective inform us about the early Natural History of Bipolar Disorder? Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l'Academie canadienne de psychiatrie de l'enfant et de l'adolescent. 2013;22(1):6-12.
42. Kim-Cohen J, Caspi A, Moffitt TE, Harrington H, Milne BJ, Poulton R. Prior juvenile diagnoses in adults with mental disorder: developmental follow-back of a prospective-longitudinal cohort. Archives of general psychiatry. 2003;60(7):709-17.
43. Kelleher I, Cannon M. Putting Psychosis in Its Place. The American journal of psychiatry. 2016;173(10):951-2.
44. American Psychiatric Association. Diagnostic criteria from DSM-IV. Washington, D.C.: The Association; 1994. xi, 358 p. p.
45. American Psychiatric Association., American Psychiatric Association. DSM-5 Task Force. Diagnostic and statistical manual of mental disorders : DSM-5. 5th ed. Washington, D.C.: American Psychiatric Association; 2013. xliv, 947 p. p.
46. Kessler RC, Avenevoli S, McLaughlin KA, Green JG, Lakoma MD, Petukhova M, et al. Lifetime co-morbidity of DSM-IV disorders in the US National Comorbidity Survey Replication Adolescent Supplement (NCS-A). Psychological medicine. 2012;42(9):1997-2010.
47. Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. The American journal of psychiatry. 2010;167(7):748-51.
48. Kotov R, Krueger RF, Watson D, Achenbach TM, Althoff RR, Bagby RM, et al. The Hierarchical Taxonomy of Psychopathology (HiTOP): A Dimensional Alternative to Traditional Nosologies. Journal of abnormal psychology. 2017.
49. Hudziak JJ, Achenbach TM, Althoff RR, Pine DS. A dimensional approach to developmental psychopathology. International journal of methods in psychiatric research. 2007;16 Suppl 1:S16-23.
50. Ivanova MY, Dobrean A, Dopfner M, Erol N, Fombonne E, Fonseca AC, et al. Testing the 8-syndrome structure of the child behavior checklist in 30 societies. Journal of clinical child and adolescent psychology : the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53. 2007;36(3):405-17.
51. Achenbach TM. Manual for the Child Behavior Checklist/4-18 and 1991 Profile. University of Vermont Department of Psychiatry1991.
52. Insel TR. Disruptive insights in psychiatry: transforming a clinical discipline. The Journal of clinical investigation. 2009;119(4):700-5.
53. Insel TR, Collins FS. Psychiatry in the genomics era. The American journal of psychiatry. 2003;160(4):616-20.
54. Cuthbert BN, Insel TR. Toward new approaches to psychotic disorders: the NIMH Research Domain Criteria project. Schizophrenia bulletin. 2010;36(6):1061-2.
55. Sanislow CA, Pine DS, Quinn KJ, Kozak MJ, Garvey MA, Heinssen RK, et al. Developing constructs for psychopathology research: research domain criteria. Journal of abnormal psychology. 2010;119(4):631-9.

7. REFERÊNCIAS

56. Pan PM, Salum GA, Gadelha A, Moriyama T, Cogo-Moreira H, Graeff-Martins AS, et al. Manic Symptoms in Youth: Dimensions, Latent Classes, and Associations With Parental Psychopathology. Journal of the American Academy of Child and Adolescent Psychiatry. 2014;53(6):625-34.
57. Caspi A, Houts RM, Belsky DW, Goldman-Mellor SJ, Harrington H, Israel S, et al. The p Factor: One General Psychopathology Factor in the Structure of Psychiatric Disorders? Clin Psychol Sci. 2014;2(2):119-37.
58. Kapur S, Phillips AG, Insel TR. Why has it taken so long for biological psychiatry to develop clinical tests and what to do about it? Molecular psychiatry. 2012;17(12):1174-9.
59. Collins PY, Patel V, Joestl SS, March D, Insel TR, Daar AS, et al. Grand challenges in global mental health. Nature. 2011;475(7354):27-30.
60. Kieling C, Herrman H, Patel V, Tyrer P, Mari JJ. A global perspective on the dissemination of mental health research. Lancet. 2009;374(9700):1500.
61. Salum GA, Mogg K, Bradley BP, Gadelha A, Pan P, Tamanaha AC, et al. Threat bias in attention orienting: evidence of specificity in a large community-based study. Psychological medicine. 2013;43(4):733-45.
62. Salum GA, Gadelha A, Pan PM, Moriyama TS, Graeff-Martins AS, Tamanaha AC, et al. High risk cohort study for psychiatric disorders in childhood: rationale, design, methods and preliminary results. International journal of methods in psychiatric research. 2015;24(1):58-73.
63. Weissman MM, Wickramaratne P, Adams P, Wolk S, Verdeli H, Olfson M. Brief screening for family psychiatric history: the family history screen. Archives of general psychiatry. 2000;57(7):675-82.
64. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. Journal of child psychology and psychiatry, and allied disciplines. 2000;41(5):645-55.
65. Fleitlich-Bilyk B, Goodman R. Prevalence of child and adolescent psychiatric disorders in southeast Brazil. Journal of the American Academy of Child and Adolescent Psychiatry. 2004;43(6):727-34.
66. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of clinical psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57.
67. Amorim P. Mini International Neuropsychiatric Interview (MINI): validation of a short structured diagnostic psychiatric interview. Rev Bras Psiquiatr. 2000;22(3):106-15.
68. Goodman R, Ford T, Simmons H, Gatward R, Meltzer H. Using the Strengths and Difficulties Questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. The British journal of psychiatry : the journal of mental science. 2000;177:534-9.
69. Cummins P, Serruys PW. The Journal Citation Reports(R) Impact Factor: annual results 2016. EuroIntervention. 2016;12(4):415-6.
70. Kupfer DJ, Frank E, Phillips ML. Major depressive disorder: New clinical, neurobiological, and treatment perspectives. The Lancet. 2012;379(9820):1045-55.
71. Dillon DG, Rosso IM, Pechtel P, Killgore WD, Rauch SL, Pizzagalli DA. Peril and pleasure: an rdoc-inspired examination of threat responses and reward processing in anxiety and depression. Depression and anxiety. 2014;31(3):233-49.
72. Koukounari A, Stringaris A, Maughan B. Pathways from maternal depression to young adult offspring depression: an exploratory longitudinal mediation analysis. International journal of methods in psychiatric research. 2016.

7. REFERÊNCIAS

73. Drakesmith M, Dutt A, Fonville L, Zammit S, Reichenberg A, Evans CJ, et al. Mediation of Developmental Risk Factors for Psychosis by White Matter Microstructure in Young Adults With Psychotic Experiences. JAMA Psychiatry. 2016;73(4):396-406.
74. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. Nat Neurosci. 2004;7(8):847-54.
75. Szyf M. Prospects for Medications to Reverse Causative Epigenetic Processes in Neuropsychiatry Disorders. Neuropsychopharmacology. 2017;42(1):367-8.
76. Stahl SM. Psychotherapy as an epigenetic 'drug': psychiatric therapeutics target symptoms linked to malfunctioning brain circuits with psychotherapy as well as with drugs. J Clin Pharm Ther. 2012;37(3):249-53.
77. Cross-Disorder Group of the Psychiatric Genomics C. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet. 2013;381(9875):1371-9.
78. Pine DS, Leibenluft E. Biomarkers With a Mechanistic Focus. JAMA Psychiatry. 2015;72(7):633-4.
79. Paulus MP. Pragmatism Instead of Mechanism: A Call for Impactful Biological Psychiatry. JAMA Psychiatry. 2015;72(7):631-2.
80. Frey BN, Andreazza AC, Houenou J, Jamain S, Goldstein BI, Frye MA, et al. Biomarkers in bipolar disorder: a positional paper from the International Society for Bipolar Disorders Biomarkers Task Force. The Australian and New Zealand journal of psychiatry. 2013;47(4):321-32.
81. Abi-Dargham A, Horga G. The search for imaging biomarkers in psychiatric disorders. Nat Med. 2016;22(11):1248-55.
82. Lazarov A, Pine DS, Bar-Haim Y. Gaze-Contingent Music Reward Therapy for Social Anxiety Disorder: A Randomized Controlled Trial. The American journal of psychiatry. 2017;174(7):649-56.
83. Drysdale AT, Grosenick L, Downar J, Dunlop K, Mansouri F, Meng Y, et al. Resting-state connectivity biomarkers define neurophysiological subtypes of depression. Nat Med. 2017;23(1):28-38.

Anexos

Termo de Consentimento

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO-HCFMUSP

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO
(OBRIGATÓRIO PARA PESQUISAS CIENTÍFICAS EM SERES HUMANOS -

RESOLUÇÃO Nº 196 - CNS)

DADOS DE IDENTIFICAÇÃO DO SUJEITO DA PESQUISA OU RESPONSÁVEL LEGAL
1. NOME: .....
DOCUMENTO DE IDENTIDADE Nº : ..... SEXO : .M □ F □
DATA NASCIMENTO: ...../...../.....
ENDEREÇO ..... Nº ..... APTO: .....
BAIRRO: ..... CIDADE .....
CEP:..... TELEFONE: DDD (.....) .....

2.RESPONSÁVEL LEGAL .....
NATUREZA (grau de parentesco, tutor, curador etc.) .....
DOCUMENTO DE IDENTIDADE :.....SEXO: M □ F □
DATA NASCIMENTO.: ...../...../.....
ENDEREÇO: ..... Nº ..... APTO: .....
BAIRRO: ..... CIDADE: .....
CEP: ..... TELEFONE: DDD (.....).....

DADOS SOBRE A PESQUISA

1. TÍTULO DO PROTOCOLO DE PESQUISA

"Esquizofrenia e Transtorno Afetivo Bipolar: identificação de estado mental de risco (EMR), caracterização de endofenótipos e impacto de intervenções para prevenção".

2 PESQUISADOR RESPONSÁVEL: EURÍPEDES CONSTANTINO MIGUEL FILHO.

CARGO/FUNÇÃO: PROFESSOR ASSOCIADO INSCRIÇÃO CONSELHO REGIONAL Nº 45280

UNIDADE DO HCFMUSP: Departamento de Psiquiatria

Investigadores executantes:

- Rodrigo Affonseca Bressan
  Professor Adjunto da Universidade Federal de São Paulo - UNIFESP.
  Inscrição no Conselho Regional no 72193 (CRM-SP)

3. AVALIAÇÃO DO RISCO DA PESQUISA:

RISCO MÍNIMO ☐ RISCO MÉDIO X

RISCO BAIXO ☐ RISCO MAIOR ☐

4.DURAÇÃO DA PESQUISA : A duração total deste projeto é prevista em 3 anos.

ANEXOS

PROPOSTA DA PESQUISA

Você está sendo convidado a participar de uma pesquisa sobre problemas de saúde mental na infância e adolescência. Essa pesquisa é uma nova fase do Estudo Epidemiológico da Saúde Mental do Escolar Brasileiro do qual você participou anteriormente e tem como objetivo ajudar os médicos e psicólogos a conhecer melhor dois dos problemas de saúde mental que podem afetar crianças e adolescentes. Ao conhecer melhor os fatores de risco para desenvolver a esquizofrenia e o transtorno afetivo bipolar esperamos poder, no futuro, planejar estratégias para prevenir seu aparecimento. Essa é a primeira vez que uma pesquisa nesse tema está sendo realizada no Brasil. A partir dos dados da primeira pesquisa foram selecionadas pessoas consideradas em risco para desenvolver estes transtornos e pessoas consideradas fora de risco. A participação de todos é muito importante para o sucesso dessa pesquisa.

Se você der sua autorização para participar da pesquisa, poderá participar de um programa de avaliação médica e neuropsicológica. Você será convidado também a preencher um questionário para responder perguntas sobre os próprios sentimentos e comportamentos no dia-a-dia. Como parte da pesquisa será convidado a fazer coleta de sangue e saliva para estudos genéticos e um exame de ressonância magnética. Você poderá optar por receber ou não receber o resultado dos exames realizados. Deixamos claro que os exames solicitados têm finalidade de pesquisa e não têm significado clínico definido, não sendo possível, a partir de nosso conhecimento atual, definir o diagnóstico de uma doença ou forma de tratamento através desses exames.

Após iniciar a pesquisa você será convidado a participar de um acompanhamento por 3 anos por uma equipe de profissionais de saúde composta por médicos psiquiatras e psicólogos. Serão realizadas avaliações a cada 6 meses durante esse período. A amostra de sangue será colhida no início do estudo. As amostras de saliva serão colhidas durante todas as visitas durante o primeiro ano da pesquisa. A ressonância magnética será feita 2 vezes, uma no início, a outra no final do estudo.

Alguns participantes do estudo serão selecionados aleatoriamente através de um sorteio para participarem com suas famílias de um programa de educação sobre os transtornos mentais. Esse programa consiste de reuniões semanais por 1 mês, seguidas de reuniões mensais por 6 meses e reuniões bimensais por mais um ano. Nessas reuniões, elementos básicos dos transtornos mentais mais comuns serão discutidos com os participantes, além de um espaço para questões e dúvidas sobre saúde mental.

Em qualquer momento do estudo você poderá solicitar sua retirada do protocolo de pesquisa sem que isso apresente qualquer conseqüência para o seguimento médico. Para tanto, somente pedimos que seja comunicado o desligamento ao corpo clínico da pesquisa. Se necessário (persistência de dúvida quanto a manutenção ou não do paciente no protocolo) será solicitado parecer da comissão de ética do Hospital das Clínicas.

RISCOS E INCONVENIÊNCIAS

As tarefas a serem realizadas para a conclusão deste projeto possuem alguns riscos e inconveniências para o participante.

1. **Coleta de amostra de sangue**. Neste procedimento pode ocorrer o aparecimento de manchas arroxeadas no local de onde o sangue foi tirado. Todos os participantes serão previamente orientados com relação a este risco e sobre os cuidados necessários caso ocorra. Além disso, raramente o local de onde foi retirada amostra de sangue pode inflamar e necessitar de cuidados locais (limpeza e pomadas) por alguns dias. Exames de sangue são necessários para dois objetivos :1. Avaliar a saúde de seu filho através de exames laboratoriais de rotina; 2. Duas das amostras de sangue (volume equivalente a 2 colheres de chá) coletadas serão utilizadas para estudos que avaliarão o envolvimento de alguns genes com o estado mental de risco.
2. **Coleta de saliva**: Não há riscos físicos envolvidos. Pode gerar angústia ou ansiedade para aqueles que considerem o procedimento desagradável.
3. **Ressonância Magnética de Crânio**: Durante o exame o único desconforto é um ruído intermitente. No entanto para amenizar o desconforto, serão fornecidos tapa-ouvidos. O exame, entretanto, pode gerar angústia ou ansiedade, pois durante o procedimento exige-se que o participante permaneça imóvel dentro da máquina. Uma das vantagens da RM é o fato de não utilizar radiações ionizantes, ao contrário de outros exames como a tomografia computadorizada. Portanto, não existem efeitos nocivos ao organismo dentro das condições normalmente utilizadas.
4. **Preenchimento dos questionários:** Lembramos que os participantes podem ficar cansados com o preenchimento dos questionários, já que demora mais ou menos uma hora para responder todas as perguntas. Também podem se sentir ansiosos por responderem perguntas sobre os próprios sentimentos e comportamentos no dia-a-dia, pois os conteúdos envolvem emoções que podem ser desagradáveis. Tentaremos minimizar estes possíveis efeitos utilizando avaliadores treinados e instrumentos curtos.

Caso você se sinta em qualquer momento desconfortável durante algum dos procedimentos listados acima, você poderá pedir para que a avaliação seja interrompida.

Caso ocorra qualquer problema relacionado ao procedimento realizado, o nosso serviço se responsabiliza pelos custos de atendimento relativos aos riscos envolvidos.

BENEFÍCIOS

Não há benefício direto para os indivíduos que participarem deste estudo além de possibilitar a identificação precoce de transtornos mentais. Isso ocorrendo possibilita a instituição mais rápida do tratamento. O potencial benefício para a sociedade é que este estudo pode aumentar o conhecimento sobre a detecção precoce e possibilidade de prevenção da Esquizofrenia e do Transtorno Afetivo Bipolar.

USO DE MATERIAL

Durante a pesquisa, amostras de sangue serão colhidas para análises genéticas. Essas amostras serão armazenadas e poderão ser utilizadas em pesquisas futuras. Toda nova pesquisa a ser feita com o material será submetida antes a aprovação do Comitê de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da Universidade de São Paulo – CAPPesq e, quando for o caso, da Comissão Nacional de Ética em Pesquisa CONEP. Caso deseje, a qualquer momento, não participar mais desta pesquisa, todos os dados genéticos serão retirados da análise.

SIGILO E PRIVACIDADE

As informações produzidas nesta tarefa serão mantidas em lugar seguro, codificadas e a identificação só poderá ser realizada pelo pessoal envolvido diretamente com o projeto. Caso o material venha a ser utilizado para publicação científica ou atividades didáticas, não serão utilizados nomes que possam vir a identificá-lo.

Em qualquer momento do estudo você poderá obter mais informações com a Dr. Rodrigo Affonseca Bressan, pelo telefone (0XX11) 5573-3599, que está apto a solucionar suas dúvidas. Você poderá solicitar informações de qualquer conhecimento significativo descoberto durante este projeto.

Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) – Rua Ovídio Pires de Campos, 225 – 5º andar – tel: 3069-6442 ramais 16, 17, 18 ou 20, FAX: 3069-6442 ramal 26 – E-mail: cappesq@hcnet.usp.br

DESPESAS E COMPENSAÇÕES

Não há despesas pessoais, ou seja, não será cobrado nada do participante em qualquer fase do estudo, incluindo exames e consultas. Também não há compensação financeira ou qualquer tipo de pagamento relacionado à sua participação. Se existir qualquer despesa adicional, ela será custeada pelo orçamento da pesquisa.

Em caso de dano pessoal, diretamente causado pelos procedimentos ou tratamentos propostos neste estudo (nexo causal comprovado), o participante tem direito a tratamento médico na Instituição.

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim, descrevendo o estudo: "Esquizofrenia e Transtorno Afetivo Bipolar: identificação de estado mental de risco (EMR), caracterização de endofenótipos e impacto de intervenções para prevenção".

Eu discuti com o Dr. **Rodrigo Affonseca Bressan** sobre a minha decisão em participar nesse estudo. Ficaram claros para mim quais são os propósitos do estudo, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que a minha participação é isenta de despesas e que tenho garantia do acesso a tratamento hospitalar quando necessário. Concordo voluntariamente na minha participação e poderei retirar o meu consentimento a qualquer momento, antes ou durante o mesmo, sem penalidades ou prejuízo ou perda de qualquer benefício que eu possa ter adquirido, ou no meu atendimento neste Serviço.

Desejo receber os resultados das avaliações a que serei submetido bem com ser esclarecido sobre seus significados (os resultados serão enviados por carta para o endereço fornecido): ( )1 SIM ( ) 2 Não (marque a opção desejada)

Assinatura do representante legal Data ___/___/___

Assinatura da testemunha Data ___/___/___

para casos de pacientes menores de 18 anos, analfabetos, semi-analfabetos ou portadores de deficiência auditiva ou visual.

(Somente para o responsável do projeto). Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste paciente ou representante legal para a participação neste estudo.

Assinatura do responsável pelo estudo Data ___/___/___

Aprovação do Comitê de Ética

UNIFESP - HOSPITAL SÃO PAULO - HOSPITAL UNIVERSITÁRIO DA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DE SINTOMAS PRECOCES DE TRANSTORNOS DE HUMOR: UMA COORTE DE ALTO RISCO

Pesquisador: PEDRO MARIO PAN NETO

Área Temática:

Versão: 1

CAAE: 65824817.3.0000.5505

Instituição Proponente: Universidade Federal de São Paulo

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.034.687

Apresentação do Projeto:

Nº CEP: 0268/2017. Os Transtornos de Humor (TH) compõem um grupo heterogêneo de quadros clínicos psiquiátricos potencialmente graves, como a Depressão, o Transtorno Bipolar (TB) e a Distimia. Os TH têm característica episódica, porém a soma dos episódios de humor confere ao quadro um curso crônico, recidivante e incapacitante. Suas alterações fisiopatológicas subjacentes são possivelmente progressivas, e a efetividade das intervenções terapêuticas disponíveis diminui conforme a evolução. Estudos preliminares indicam que os quadros de humor podem ter início com sintomas subsindrômicos e que uma fase pré-clínica pode ser identificada. Contudo, poucos estudos longitudinais avaliaram as principais manifestações psicopatológicas precoces desses transtornos, particularmente do Transtorno Bipolar (TB), e seu poder preditivo como marcador de risco para o desenvolvimento da doença.

Objetivo da Pesquisa:

Objetivo primário: identificar fatores de risco e de proteção, a partir de diferentes métodos, associados longitudinalmente às diferentes trajetórias de TH na infância e adolescência. Objetivos secundários: comparar os níveis de psicopatologia geral e comorbidades entre indivíduos em risco e indivíduos não afetados. Comparar os níveis de psicopatologia geral e de comorbidades dos pais biológicos dos indivíduos em risco com os pais de controles saudáveis e sua influência na

Endereço: Rua Botucatu, 572 1º Andar Conj. 14
Bairro: VILA CLEMENTINO **CEP:** 04.023-061
UF: SP **Município:** SAO PAULO
Telefone: (11)5571-1062 **Fax:** (11)5539-7162 **E-mail:** secretaria.cepunifesp@gmail.com

psicopatologia do indivíduo índice. Identificar agrupamentos latentes de sintomas subsindrômicos de TH como fatores de risco associados ao desenvolvimento do quadro sindrômico. Identificar agrupamentos latentes de variáveis de neuropsicologia e de neuroimagem como fatores de risco associados ao desenvolvimento dos TH. Hipóteses: a principal hipótese consiste na existência de um conjunto de variáveis clínicas, neuropsicológicas, e de neuroimagem que pode predizer a transição de um estado mental de risco para o diagnóstico psiquiátrico de um TH. As hipóteses secundárias do estudo são: 1) Existe um gradiente de sintomas de Mania e de Depressão, que vai da ausência até o preenchimento completo dos critérios diagnósticos, associado a um comprometimento funcional proporcional à gravidade dos mesmos, que evoluiu com o decorrer do tempo de forma dose dependente. 2) A gravidade e a persistência de sintomas subsindrômicos de Mania e de Depressão são preditores para o desenvolvimento de TH, principalmente de TB. 3) Existe uma associação entre sintomas subsindrômicos, história familiar de transtornos mentais e o desenvolvimento de TH. 4) Existe uma associação entre sintomas subsindrômicos, perfis neuropsicológicos específicos e o desenvolvimento de TH. 5) Há marcadores de neuroimagem capazes de mediar a associação entre sintomas subsindrômicos e o desenvolvimento de TH.

Avaliação dos Riscos e Benefícios:

Segundo o pesquisador: Riscos: o presente estudo analisará dados previamente coletados pelo INPD. Portanto, não será realizada nenhuma avaliação direta de pacientes. Para a coleta de dados do INPD, foram priorizadas as condições estabelecida. O estudo envolveu somente riscos associados ao questionamento e obtenção de informações, além de riscos médios para a captação de imagens através do método de Ressonância Magnética. Para diminuir possíveis efeitos negativos, como o cansaço durante a entrevista, os pesquisadores priorizaram instrumentos sucintos e objetivos. Ainda, os protocolos para aquisição da neuroimagem foram reduzidos e adequados ao bem-estar dos sujeitos e de seus familiares. Antes de entrar para o exame, os sujeitos participavam de uma atividade lúdica por cerca de 30 minutos, que explicava todos os passos do procedimento através de simulações com técnicas lúdicas. Digno de nota, tendo em vista a taxa de sucesso encontrada a partir desse procedimento com técnicas lúdicas, aventa-se a implementação de protocolo semelhante para exames de rotina em alguns serviços pediátricos. O grupo de pesquisa pode garantir confidencialidade das informações. Todos os participantes assinaram o Termo de Consentimento Livre e Esclarecido previamente ao estudo, estando livres para decidir em qualquer momento sobre sua descontinuidade. A equipe manteve um canal aberto de contato com os participantes durante todo o tempo da pesquisa. Indivíduos com alterações importantes no exame de neuroimagem ou alterações psicopatológicas significativas foram

avaliados por clínicos e encaminhados para seguimento e atendimento especializado. Benefícios: a avaliação longitudinal de sintomas subsindrômicos de TH, assim como fatores possivelmente associados ao seu desenvolvimento, é fundamental para o entendimento das diferentes evoluções do quadro. O estudo prospectivo dos sintomas de Depressão, de Mania, e dos constructos latentes previamente encontrados poderão trazer novas informações sobre a existência de um grupo de risco para os TH. Ainda, como a grande maioria dos estudos conduzidos sobre o tema é proveniente de países da América do Norte e da Europa, a avaliação desses sintomas em diferentes contextos socioeconômicos é necessária para validar seu constructo psicopatológico nessa faixa etária. Até o presente momento, nenhum estudo avaliou longitudinalmente a distribuição de sintomas de Mania e poucos estudos avaliaram a distribuição dos sintomas de Depressão em amostras populacionais de países em desenvolvimento. A associação das dimensões de sintomas com fatores de risco já conhecidos, tais como história familiar, estresse ambiental precoce, endofenótipos neuropsicológicos, e alterações estruturais e funcionais de neuroimagem pode fornecer evidências para a validade do construto de estados precoces de risco. As dimensões e as classes latentes podem representar um avanço para a clínica, pois ajudariam o clínico a identificar qual grupo de sintomas tem melhor capacidade discriminativa e maior associação com prejuízos. Podem, ainda, constituir uma nova perspectiva para a abordagem de estados mentais de risco nos TH, de forma análoga ao que ocorreu no campo das psicoses, agrupando e determinando a relevância clínica de sintomas subsindrômicos. Por fim, os resultados podem servir como parâmetro inicial para o planejamento e delineamento de estudos de intervenção, com o intuito de tratar sintomas nas fases iniciais dos TH ou, eventualmente, até prevenir sua evolução para um quadro que alcance o limiar clínico. Medidas mais precoces poderiam usar elementos com menor nível de intervenção e, consequentemente, menos efeitos colaterais e menores riscos.

Comentários e Considerações sobre a Pesquisa:

Trata-se de estudo com o objetivo acadêmico de Doutorado, vinculado ao PROGRAMA DE PÓS-GRADUAÇÃO EM PSIQUIATRIA, Departamento de Psiquiatria, Campus São Paulo. Orientador: Prof. Dr. Rodrigo Affonseca Bressan Metodologia a ser empregada: o presente estudo está inserido na fase de seguimento do Projeto Prevenção, composto pelos projetos 2 a 5 do Instituto Nacional da Psiquiatria do Desenvolvimento (INPD). O INPD faz parte dos Institutos de Ciência e Tecnologia & Inovação (INCTs) do CNPq, tendo recebido aprovação no edital de 2008. Trata-se de um estudo de metodologia longitudinal de coorte, utilizando dados já coletados do projeto INPD. O INPD avaliou aproximadamente 10.000 crianças entre 6 a 12 anos em sua primeira etapa em 2009. Destes, foram escolhidos 2.500 indivíduos para avaliação domiciliar e escolar. A amostra de 2.500

indivíduos foi composta por 1.000 indivíduos aleatoriamente escolhidos e 1.500 indivíduos com altos índices de psicopatologia familiar e individual identificados pelo instrumento de triagem (FHS). Os 2.500 indivíduos selecionados foram avaliados em 2010 através de entrevistas psiquiátricas padronizadas e de investigação abrangente de fatores sociodemográficos e clínicos. Uma sub amostra realizou exames de neuroimagem através de Ressonância Magnética, em equipamentos de 1,5 Tesla de campo magnético. As sequências utilizadas foram (a) T1-weighted 3D alta resolução; (b) Imagem por tensão de difusão (DTI); (c) conectividade intrínseca funcional em descanso (Resting State fMRI); (d) MT ON/OFF. A duração total do escaneamento foi de 28 minutos. A avaliação neuropsicológica consistia nos seguintes testes: Twochoice reaction time (Hogan et al., 2005); Conflict control task (Hogan et al., 2005), Go/No-Go (Bitsakou et al., 2008).Attention Attention network task (Fan et al., 2002), Dot-probe task (Mogg et al., 1997), Time anticipation 400 ms and 2000 ms (Toplak and Tannock, 2005), Duration discrimination task (Toplak et al., 2003), Delay reaction task (Sonuga-Barke and Taylor, 1992), Choice delay task (Sonuga-Barke and Taylor, 1992), Wechsler Intelligence Scale for Children (vocabulary and block) (Wechsler, 2002). Para detalhes da metodologia, ver Salum et al. (2015). Dentre os indivíduos avaliados em 2010, 80,05% foram localizados e aceitaram participar de uma nova fase de coleta de dados, que consistia em protocolo de pesquisa semelhante. Esta fase ocorreu em 2013, quando os sujeitos apresentavam idade entre 9 e 16 anos. Foram incluídas medidas para avaliação de comportamentos do adolescente na avaliação de 2013, quais sejam: .Questionário sobre uso de substâncias ilícitas, álcool e tabaco (DAWBA) .Detalhamento sobre tratamentos clínicos e de saúde mental nos últimos três anos .Avaliação de fatores de risco ambientais e escolares nos últimos três anos Critérios de inclusão: Indivíduos que completaram a primeira (2010) e a segunda (2013) fase do Projeto Prevenção/INPD; Prover consentimento adequado - de acordo com o TCLE e TAE em anexo Os principais critérios de inclusão da primeira fase foram: matrícula regular em uma das 57 escolas cadastradas no projeto; pai biológico capaz de responder à entrevista; apresentar entre 6 e 12 anos completos no momento do contato com a equipe de pesquisa. Indivíduos que completaram a primeira fase do Projeto Prevenção. Critérios de exclusão: incapacidade de entrar em contato com o indivíduo para a realização da segunda fase em 2013 através dos dados fornecidos na primeira fase do Projeto Prevenção/INPD; recusa a participar da fase de reavaliação de três anos em 2013. Os principais critérios de exclusão da primeira fase foram: presença de doença orgânica grave; ausência de pelo menos um pai biológico capaz de responder à entrevista. Avaliação psicopatológica: incluiu os seguintes instrumentos, utilizados na primeira fase do projeto: avaliação diagnóstica: a. Crianças e adolescentes: DAWBA (Development and Well-Being

Assessment) b. Pais biológicos dos indivíduos pesquisados: MINI (MiniInternational Neuropsychiatric Interview). Avaliação geral de psicopatologia: CBCL (Child Behavior Checklist) (Strengths and difficulties questionnaire). Questionário sobre uso de substâncias ilícitas, álcool e tabaco (DAWBA). Detalhamento sobre tratamentos clínicos e de saúde mental nos últimos três anos (SACA). Avaliação de fatores de risco ambientais e escolares nos últimos três anos; avaliação sobre estigma em saúde mental. Análise Estatística.

Considerações sobre os Termos de apresentação obrigatória:

Documentos obrigatórios apresentados: Folha de Rosto FolhadeRostoassinadamar17.pdf; Projeto Detalhado / Brochura Investigador Projeto_CEPu_sub.pdf TCLE /TCLE_sub.pdf; TCLE TAE_sub.pdf (estudo anterior) Declaração de Pesquisadores cartaanuencia.pdf. Outros documentos: CadastroCEPUNIFESPPanSigned.pdf; O pesquisador propõe dispensa do TCLE. Justificativa: os dados já foram coletados conforme descrito na metodologia, e as análises da presente proposta estão englobadas no projeto "ESQUIZOFRENIA E TRANSTORNO AFETIVO BIPOLAR: IDENTIFICAÇÃO DE ESTADO MENTAL DE RISCO (EMR), CARACTERIZAÇÃO DE ENDOFENÓTIPOS E IMPACTO DE INTERVENÇÕES PARA PREVENÇÃO" e em seus subsequentes relatórios parciais, submetidos desde o início pelo orientador da presente proposta, prof Rodrigo Bressan. O projeto e os relatórios parciais foram aprovados pelo comitê de ética CEAPESQ-USP sob o CAAE 0009.1.015.015-09. Não serão realizadas novas avaliações dos sujeitos de pesquisa.

Recomendações:

Nada consta.

Conclusões ou Pendências e Lista de Inadequações:

Prezados pesquisadores, atenção à Legislação: corrigir à norma aplicável. A Resolução Normativa 196/96 foi revogada e atualmente a norma que rege a pesquisa com seres humanos é a Resolução Normativa 466/12 do CNS/MS.

Considerações Finais a critério do CEP:

O CEP informa que a partir desta data de aprovação, é necessário o envio de relatórios parciais (anualmente), e o relatório final, quando do término do estudo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_847900.pdf	16/03/2017 17:44:54		Aceito

Outros	CadastroCEPUNIFESPPanSigned.pdf	16/03/2017 17:44:04	PEDRO MARIO PAN NETO	Aceito
Declaração de Pesquisadores	cartaanuencia.pdf	09/03/2017 12:40:20	PEDRO MARIO PAN NETO	Aceito
Folha de Rosto	FolhadeRostoassinadamar17.pdf	09/03/2017 12:28:00	PEDRO MARIO PAN NETO	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEPu_sub.pdf	16/02/2017 01:07:02	PEDRO MARIO PAN NETO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TAE_sub.pdf	16/02/2017 00:57:19	PEDRO MARIO PAN NETO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_sub.pdf	16/02/2017 00:57:03	PEDRO MARIO PAN NETO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 26 de Abril de 2017

Assinado por:
Miguel Roberto Jorge
(Coordenador)

APÊNDICE